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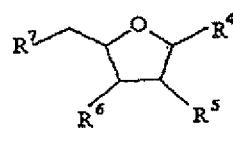
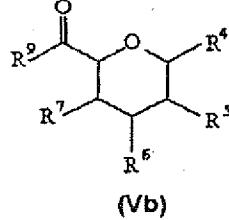
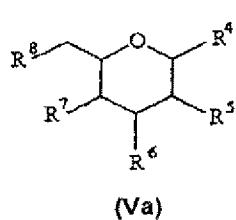
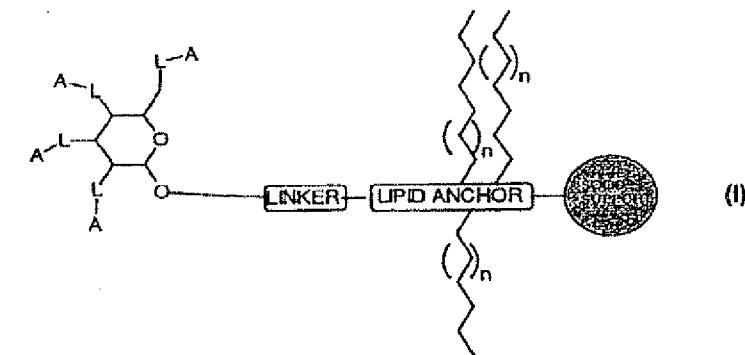
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(54) Title: IMMUNOADJUVANT SYSTEMS



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(57) Abstract: This invention relates to compounds of general formula I, in which S is a carbohydrate core matrix; a is a pharmaceutically active moiety; L is a linker a covalent bond; n is an integer of 2 to 19; and R¹ is hydrogen, thioalkyl or X-Z in which Z is a lipophilic anchor and X is a linker or covalent bond. Formulae Va, Vb and Vc where R¹-R⁹ are as defined in the specification; and delivery systems for pharmaceutically active agents.

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IMMUNOADJUVANT SYSTEMS

This invention relates to delivery systems for pharmaceutically-active agents. In particular, the 5 invention relates to compounds comprising a carbohydrate moiety and optionally a lipid moiety, which are useful as delivery agents.

It is well recognised that synthetic peptides can induce antibodies which are reactive with their cognate 10 sequences in the native proteins, that is, the synthetic peptide and the native protein present the same epitope. Specific antibodies are useful as reagents in a wide variety of investigations. Furthermore, peptide antigens, made by peptide synthesis techniques known in the art, are 15 useful for producing immunogens, in immunoprophylaxis and in affinity purification of proteins, antibodies, or other molecules.

Advances in the areas of biotechnology, 20 biochemistry and peptide synthesis have led to the availability of large quantities of pure, potent and highly specific peptides, which can be used to generate 25 antibodies. However, cognate peptide sequences may not in themselves provide suitable antigens. In many cases protein immunogens are glycosylated, and the glycosylation pattern itself may confer higher antigenicity than the cognate peptide sequence.

Small peptide molecules or saccharides may not 30 themselves be of sufficient molecular weight to be immunogenic at all or to a sufficient degree, but can be rendered immunogenic or more immunogenic by conjugation to a carrier molecule, for example a protein or a synthetic polymer. Proteins such as bovine serum albumin (BSA), keyhole limpet haemocyanin (KLH) or diphtheria toxin (DT), are widely used for this purpose.

35 Although the conjugated product is antigenic, it comprises a large number of epitopes other than that associated with the synthetic peptide of interest. Di

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Marchi et al (1986) describe a synthetic peptide comprising two immunologically significant regions of a virus coat protein, joined by a spacer which has adequate molecular weight to induce an immune response. However, the spacer 5 region itself can act as an antigenic epitope, which is undesirable.

Tam (1988) described an approach to increasing the molecular weight of synthetic peptide antigens, called the multiple antigen peptide system (MAP). The MAP 10 comprises a core matrix formed of a low number (n) of sequential levels of dendritically-linked trifunctional amino acids, in practice lysine molecules. The core matrix has 2^n terminal functionalities, in practice amine 15 functionalities, each of which can be conjugated with a synthetic peptide. The MAP was synthesized by forming the core matrix using a conventional stepwise solid phase procedure. Subsequent levels of the core were formed by similar steps. A preformed synthetic peptide antigen molecule was joined to each terminal amine functionality 20 via a triglycyl linker, followed by cleavage from the resin by conventional means.

Unfortunately, the progress in antigen production has not been matched by a similar rate of progress in vaccine adjuvant technology, and currently available 25 vaccine adjuvants are associated with various problems, including toxicity, hypersensitivity and short-term effects. The adjuvant, by its nature, tends to be an irritant or toxic compound. The only adjuvants approved for broad human use are aluminum hydroxide and aluminum 30 phosphate. An adjuvant being developed by Corixa Corporation is a derivative of the lipid A molecule found in Gram-negative bacteria, and is considered to be one of the most powerful immunostimulants known. However, it is not yet approved for use in humans.

35 Traditional vaccine approaches for eliciting immune protection against pathogenic organisms, particularly bacteria and viruses, involve the use of

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attenuated pathogens, alone or with an aluminum hydroxide adjuvant. While these approaches are effective, they carry a small but significant risk of causing disease as a result of reversion of the pathogen to virulence.

5 More modern vaccines use genetic engineering to yield safer immunogens in which one or more proteins essential for virulence is deleted so that the pathogen is theoretically incapable of reversion to virulence. These have the potential for use against a wider range of
10 diseases. However, these new vaccines frequently do not stimulate a sufficiently strong immune response to provide protection from infection. In most cases, the synthetic peptides require immunopotentiation if they are to be effective vaccines. Furthermore, there are formidable
15 challenges confronting the oral delivery of vaccines, including attack by enzymes, instability in the gut, short retention times, and barriers to absorption and transport. It is evident that traditional methods are unsatisfactory, requiring multi-component formulations.

20 Systems in which a polylysine dendrimer is used as part of the core structure of an antigen-dendrimer system are known in the art. When employing polylysine dendrimers for an antigen or pharmaceutically active molecule, 2^n coupling points, where n refers to the number
25 of levels of lysines in the dendrimer, may be generated at each successive branching point. In contrast, when using carbohydrates, up to 4^n coupling points, where n refers to the number of monosaccharide units in the carbohydrate, may be generated as each successive monosaccharide is
30 coupled. The higher density using fewer units which is possible when using a carbohydrate core leads to a greater propensity for synergistic effects and cooperative binding.

35 Toth et al have developed a system in which a lipidic adjuvant is chemically conjugated to the antigen of interest. This system combines the MAP system with a lipidic anchor moiety to form a Lipid-Polylysine Core-Peptide (LCP) (Toth et al., 1993; Toth and Gibbons, British

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Patent Application No. 9215780.9 (24 July, 1992); Toth and Gibbons, European Patent Application No. 93917902.4). In the LCP system, lipid amino acids (LAAs) are incorporated as a lipidic anchor moiety at the C-terminal of a

5 polylysine-peptide system. Thus the carrier, adjuvant and antigen are contained in the same molecule. This molecular entity can be readily synthesised in a single reaction vessel, by step-wise solid phase methods, without requiring the isolation of intermediates. Properties such as

10 molecular weight, charge, lipophilicity, targeting moieties, and radiolabels can readily be varied in the LCP system, by modifying these variables in the LAA. Considerable control over stability, loading, permeability, antigenicity, *in vivo* behaviour and other factors is also

15 possible. Promising immunological data were obtained in preliminary studies using the LCP system via the parenteral route. For example, the immunogenicity was found to be much greater for synthetic peptides from Group A Streptococci (Pruksakom et al. 1994), foot and mouth

20 disease virus (FMDV) (France et al., 1994) and *Chlamydia trachomatis* (Zong et al., 1995) incorporated into the LCP system, than when given with Freund's adjuvant (Toth et al., 1995). It has also been demonstrated that the long alkyl chains of the LAAs are capable of protecting a labile

25 synthetic peptide from enzymatic attack (Toth et al., 1994). Thus the effectiveness of a vaccine, adjuvant and particulate carrier present within the same molecule for delivery of pharmaceutically active moieties has been demonstrated.

30 We have now found that the LCP technology can be significantly improved by replacing the polylysine adjuvant/core structure of the carrier system with a monosaccharide or oligosaccharides.

35 SUMMARY OF THE INVENTION

According to the present invention there is provided a compound of general formula I

- 5 -

$(A-L)_n-S-R'$

I

5

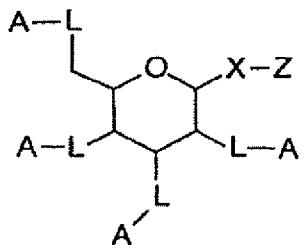
in which S is a carbohydrate core matrix;
A is a pharmaceutically active moiety;
L is a linker or covalent bond;
n is an integer of 2 to 19; and
10 R' is hydrogen or -X-Z in which Z is a lipophilic anchor and X is a linker or covalent bond.

The carbohydrate core matrix S may be a natural or synthetic monosaccharide or an oligosaccharide preferably having 1 to 6 monosaccharide moieties. Non 15 limiting examples include, glucose, glucosamine, galactose, mannose, glucuronic acid, iduronic acid, idose, fucose, galactosamine, sucrose, fructose, maltose, lactose, lactosamine, globotriose, globotetraose, sialyl lewis X, lewis X, lewis Y, lewis b tetrasaccharide, lewis a, sialyl 20 lewis a, chitobiose, chitotriose, chitotetraose, chitopentaose, chitohexaose, blood group A trisaccharide, blood group b trisaccharide, blood group H disaccharide, blood group H II trisaccharide, galabiose, T antigen, $\alpha 1,3$ galactobiose, Gal $\alpha 1,3$ Gal $\beta 1,4$ GlcNAc, Galili, 25 pentasaccharide, mannobiose, 3'-sialyllactose, 6'-sialyllactose, sialyl lacto-N-tetraose, lacto-N-tetraose, lacto-N-neotetraose, lacto-N-hexaose, lacto-N-neohexaose, cellobiose, cellopentaose, cellotetraose, cellotriose, α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin, ribose, 30 arabinose, trigalacturonic acid, maltotriose which may be branched or linear.

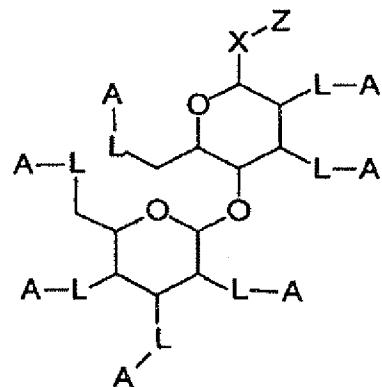
It will be understood that the value of n will depend on the number of available carbohydrate functional groups on the core matrix S.

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Typically, the available functional groups on S will be free hydroxyl, amino and/or carboxylate groups. For example, where S is a monosaccharide, n is an integer from 2 to 5 (Ex I); when S is a disaccharide, n is an integer from 2 to 7 (Ex II); where S is a trisaccharide, n is an integer from 2 to 10, and so on.



Ex I



Ex II

The pharmaceutically-active moiety A may be selected from synthetic or natural peptides, proteins, mono- or oligosaccharides, sugar-amino acid conjugates, sugar-peptide conjugates, drugs, pro-drugs or drug like molecules. Also included for moiety A is antibodies or are antigen binding fragments of whole antibody, wherein the fragments retain the binding specificity of the whole antibody molecule. The binding fragments include, for example, Fab, F(ab')₂, and Fv fragments. Binding fragments can be obtained using conventional techniques, such as proteolytic digestion of antibody by papsin or pepsin, or through standard genetic engineering techniques that are well known in the art.

The pharmaceutically-active moiety may, for example, serve as an antigen or antigenic determinant, toxin, ligand, drug or pro-drug, and may be either antigenic or non-antigenic when taken alone. As at least

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two pharmaceutically active moieties are present, it will be appreciated that they may be the same or different. The pharmaceutically-active moieties A are attached to the core matrix either directly or via the linker L using coupling methods known in the art and compatible with the functionalities on the linker L.

5 The linker L may be alkyl, alkenyl, alkynyl, heteroalkyl, arylalkyl, or heteroarylalkyl of 3 to 12 atoms in length, which may be optionally substituted, branched or linear. Examples of substituents include functional groups suitable for conjugation with a pharmaceutically active moiety, such as CO₂H, NH₂, SH, OH, halo or N=C=O. As at least two linkers L are present, it will be appreciated that they may be the same or different. L may be attached 10 to the core matrix S by one or more linkage methods known in the art, such as, for example, ether, ester, or amide linkages.

15 The linker X may be:

a) a monosaccharide or an oligosaccharide 20 having 1 to 4 monosaccharide moieties, which modifies the physicochemical properties, such as water solubility, targets the compound to specific sites such as mannose, targets an active uptake mechanism such as glucose transport system and/or modifies the immune response;

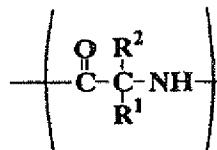
b) a spacer of up to 14 atoms which separates 25 the lipophilic anchor Z from the core matrix S; or

c) a peptide or amino acid which modifies the physicochemical properties of the compound, or which provides suitable spacing between the lipophilic anchor Z 30 and the core matrix S.

The spacer (b) may be an alkyl, alkenyl, alkynyl, heteroalkyl, arylalkyl or heteroarylalkyl, which may be optionally substituted. Suitable spacers include polyethyleneglycol or polyglycine.

35 The lipophilic anchor Z may be 1 to 6 lipoamino acids of general formula II

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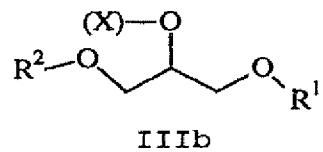
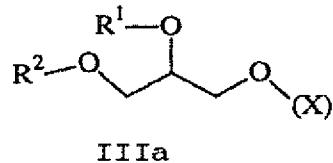
II

in which each of R^1 and R^2 are the same or different and
5 selected from:

hydrogen or an alkyl or alkenyl having 4 to 24 carbon atoms, which may be optionally substituted with substituents that do not significantly adversely affect the lipophilic nature of the anchor,

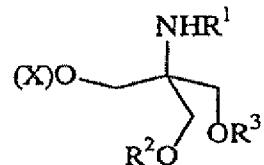
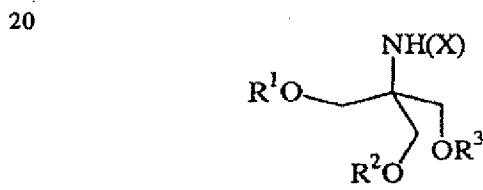
10 with the proviso that both R^1 and R^2 are not hydrogen;

(b) a glycerol based lipid of the general formula IIIa or IIIb



15 in which R^1 and R^2 are as defined in formula II above and X is the linker defined in formula I above;

(c) a trishydroxymethylmethylamine-based lipid of general formula IVa or IVb



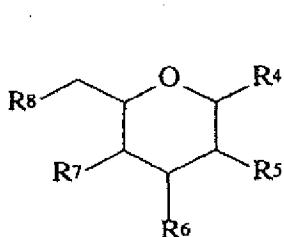
25 in which R^1 , R^2 and R^3 are the same or different and selected from hydrogen or a group having 4 to 24 carbon atoms which may be optionally substituted, with

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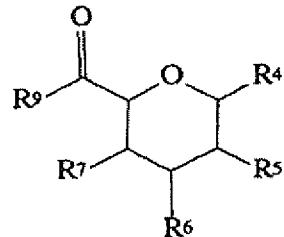
substituents do not significantly adversely affect the lipophilic nature of the anchor; and

X is the linker as defined in formula I above, with the proviso that only one of R¹, R² and R³ can be hydrogen;

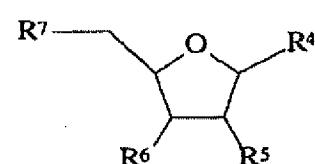
(d) a compound of general formula Va, Vb or Vc



Va



Vb



Vc

in which the groups R⁴, R⁵, R⁶, R⁷ and R⁸ are the same or different and selected from O(X), NH(X), OH, OW, N-(C=O)-W and NH₂;

W is a alkyl or alkenyl group having 4 to 24 carbon atoms which may be optionally substituted with substituents do not significantly adversely affect the lipophilic nature of the anchor, (X) is the linking group to the sugar core,

with the provisos that at least two of the groups R⁴ to R⁸ must be OW and only one of the groups may be O(X) or NH(X).

in which R⁹ is selected from OW, (X), O(X), NH-W, NH-(X), OH or NH₂ where W is as defined above, (X) is the linking group to the sugar core, with the proviso that R⁹ may only be (X), O(X) or NH-(X) if none of R⁴, R⁵, R⁶, R⁷ or R⁸ are O(X) or NH(X).

25 additionally, R⁴ may be thioalkyl.

The compounds of formulae Va, Vb and Vc represent a new class of lipophilic anchors and therefore form another aspect of the invention.

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Suitable substituents in (a), (b), (c) and (d) include halo, hydroxy and thiol. It may be convenient to include substituents so as to confer suitable solvent solubility properties upon the system.

5 The lipoamino acids in (a) may be coupled sequentially, or may be interspersed with up to 4 other amino acid spacers such as serine or arginine which modify the properties such as solubility of the lipophilic anchor.

The term "alkyl" denotes straight chain, branched 10 or cyclic alkyl, preferably C₁₋₃₀alkyl or cycloalkyl. Examples of straight chain and branched alkyl include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, amyl, isoamyl, sec-amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, hexyl, 4-methylpentyl, 15 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, heptyl, 5-methylhexyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 20 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, octyl, 6-methylheptyl, 1-methylheptyl, 1,1,3,3-tetramethylbutyl, nonyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-methyloctyl, 1-, 2-, 3-, 4- or 5-ethylheptyl, 1-, 2- or 3-propylhexyl, decyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- and 8-methylnonyl, 1-, 2-, 3-, 4-, 5- or 6-ethyloctyl, 1-, 2-, 3- or 4-propylheptyl, undecyl 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8- or 9-methyldecyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-ethylnonyl, 1-, 2-, 3-, 4- or 5-propyloctyl, 25 1-, 2- or 3-butylheptyl, 1-pentylhexyl, dodecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-methylundecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5- or 6-propylnonyl, 1-, 2-, 3- or 4-butyloctyl, 1-2 pentylheptyl and the like. Examples of cyclic alkyl include mono- or 30 35 polycyclic alkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl and the like.

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The term "alkenyl" denotes groups formed from straight chain, branched or cyclic alkenes including ethylenically mono-, di- or poly-unsaturated alkyl or cycloalkyl groups as defined above, preferably C₂-₂₀ alkenyl.

5 Examples of alkenyl include vinyl, allyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methyl-cyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, cyclooctenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 10 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,3,5-cycloheptatrienyl and 1,3,5,7-cyclooctatetraenyl.

15 The term "alkynyl" denotes groups formed from straight chain, branched, or mono- or poly- or cyclic alkynes. Examples of alkynyl include ethynyl, 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, 5-hexynyl, 10-undecynyl, 4-ethyl-1-octyn-3-yl, 7-dodecynyl, 9-dodecynyl, 10-dodecynyl, 3-methyl-1-dodecyn-3-yl, 2-tridecynyl, 11-tridecynyl, 3-tetradecynyl, 7-hexadecynyl, 3-octadecynyl and the like.

20 The term "aryl" used either alone or in compound words such as "heteroaryl" denotes single, polynuclear, conjugated and fused residues of aromatic hydrocarbons or aromatic heterocyclic ring systems. Examples of aryl include phenyl, biphenyl, terphenyl, quaterphenyl, phenoxyphenyl, naphthyl, tetrahydronaphthyl, anthracenyl, dihydroanthracenyl, benzanthracenyl, dibenzanthracenyl, 25 phenanthrenyl, fluorenyl, pyrenyl, indenyl, azulenyl, chrysenyl, pyridyl, 4-phenylpyridyl, 3-phenylpyridyl, thienyl, furyl, pyrryl, pyrrolyl, furanyl, imadazolyl, pyrrolydiny, pyridinyl, piperidinyl, indolyl, pyridazinyl, 30 pyrazolyl, pyrazinyl, thiazolyl, pyrimidinyl, quinolinyl, isoquinolinyl, benzofuranyl, benzothienyl, purinyl, 35 quinazolinyl, phenazinyl, acridinyl, benzoxazolyl, benzothiazolyl and the like. Preferably, the aromatic

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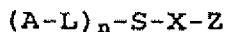
heterocyclic ring system contains 1 to 4 heteroatoms independently selected from N, O and S and containing up to 9 carbon atoms in the ring.

In this specification "optionally substituted"

5 means that a group may or may not be further substituted with one or more groups selected from alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, carboxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, 10 nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, azido, amino, alkylamino, alkenylamino, alkynylamino, arylamino, benzylamino, acylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, acyloxy, aldehydo, alkylsulphonyl, arylsulphonyl, sulphonylamino, 15 alkylsulphonylamino, arylsulphonylamino, alkylsulphonyloxy, arylsulphonyloxy, heterocyclyl, heterocycloxy, heterocyclylamino, halo(heterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, sulfonic acid, alkylthio, arylthio and acylthio.

20 The term "halo" denotes fluorine, chlorine, bromine or iodine.

In one embodiment, the compound of formula I is of the formula Ia



25

Ia

in which S, A, L, n, X and Z are as defined in formula I above.

30 In one preferred embodiment of formula Ia, Z, X, L and A are as defined in formula I;

S is a mono- or disaccharide example, such as, glucose, galactose, lactose, glucosamine, or glucuronic acid; and

35 n is an integer from 2 to 7; for example in the case of a monosaccharide core matrix n is an integer from 2

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to 5, and in the case of a disaccharide core matrix, n is an integer from 2 to 7.

In a second preferred embodiment of formula Ia, X and A are as defined for formula I, Z is 2 to 4 lipoamino acids of general formula II as defined above, in which R¹ and R² are either H or a linear alkyl or alkenyl group having 6 to 20 carbon atoms;

S is a mono- or disaccharide such as glucose, galactose, lactose, glucosamine or glucuronic acid; L is attached to S by an ether or amide bond, and each L is an optionally substituted alkyl or optionally substituted heteroalkyl group of 3 to 12 atoms in length; and

n is an integer from 2 to 7; for example in the case of a monosaccharide core matrix n is an integer from 2 to 5 and in the case of a disaccharide core matrix, n is an integer from 2 to 7.

In a third preferred embodiment of formula Ia, Z is a compound of general formula Va, Vb, Vc as defined above.

In another embodiment, the compound of formula I is of the formula Ib

(A-L)_n-S

25

Ib

in which A, L and S are as defined in formula I above; and

n is an integer of 2 to 11.

30 Preferably the core matrix S is a mono or disaccharide; the linkers L are the same or different and selected from linear alkyl or heteroalkyl groups of 3 to 12 atoms in length which may be optionally substituted and are attached to the core matrix S by an ether or amide bond; 35 and

the pharmaceutically active moieties A are the same and are selected from the group consisting of

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synthetic or natural peptides, proteins, mono or oligosaccharides, sugar-amino acid conjugates, sugar-peptide conjugates, drugs, pro-drugs, and drug-like molecules.

5 The invention also provides a method for the preparation of a compound of formula I, comprising the steps of

- (a) attaching the linker L to the core matrix S;
- (b) attaching a pharmaceutically active moiety A 10 to the linker L; and
 - when R' is -X-Z
 - c) linking the lipophilic anchor Z and the core matrix S either by (i) the reaction of a glycosylamine with the carboxylate terminus or amine of the lipophilic anchor,
- 15 (ii) the reaction of a glycosylamine with the carboxylate terminus of the lipophilic acid and linker X to form a peptide bond, (iii) glycosylating the core matrix S via the lipophilic anchor Z or the linker X to form an N, O, or S glycosidic bond and/or (iv) reacting a glycosyl carboxylate 20 either directly or via a linker X with an amine of the lipophilic anchor Z to form a peptide bond.

The person skilled in the art will be aware of suitable methods of performance all of steps (a) to (c).

25 The method may be performed in solution, on a solid support or by a combination of solution phase and solid supported steps.

Typically, the linker L is attached to the matrix core S by the formation of an ether bond in solution. The matrix core S - linker L conjugate may then be immobilised 30 onto a solid support or reacted in solution with a pharmaceutically active moiety A to form a covalent linkage between the pharmaceutically active moiety A and the linker L. This covalent bond may be an amide, disulfide, ester, urea or other suitable covalent bond known in the art.

35 In step (c)(i) and (ii), a variety of methods known in the art may be used. For example, the linkage may form a urea or thiourea bond between the lipophilic anchor

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or linker and the core matrix, or a linker such as glutaric acid is coupled to form peptide bonds to both the core matrix and the lipophilic anchor.

In one embodiment the formation of the linkage 5 between the matrix core and the lipophilic anchor, with or without a spacer, is carried out on a solid support.

In a second embodiment, the compound of formula I is built up by a step-wise procedure from the respective components on a solid support.

10 In a third aspect the invention provides a composition comprising a compound according to the first or the second aspect of the invention, together with a pharmaceutically-acceptable carrier.

15 In a fourth aspect the invention provides a method of treating or preventing a disease comprising the step of administering to a subject in need thereof a therapeutically-effective amount of a compound according to the first or the second aspect of the invention, together with a pharmaceutically-acceptable carrier.

20 For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

25 Figure 1 is a schematic representation of a representative lipid-sugar immunogen construct according to the invention, in which A is an antigenic epitope, L is a linking arm, and n refers to the length of the lipid chain.

30 In a preferred aspect the present invention relates to a new antigen delivery system, the lipid-sugar- immunogen, wherein lipid anchors comprised of lipid-aminoacids (LAA), liposaccharides, lipoglycerols, or similar structural motifs, are coupled via a linker, which may be peptidic, polyethyleneglycolic, aryl or otherwise, to a carbohydrate core structure. The carbohydrate core 35 structure is composed of a mono or oligosaccharide, in which some, but not necessarily all, carbohydrate functional groups are coupled, either directly or via a

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linking or spacer arm, to a pharmaceutically active molecule of interest. The structure of the lipid-sugar-immunogen is schematically illustrated in Figure 1. The entire system may be synthesised while coupled to a solid 5 support.

Cheap, commercially available sugars, such as maltose or lactose, can be employed in the core structure with great effectiveness. An added advantage of employing carbohydrates as part of the core structure of a lipid- 10 immunogen system, is their general biocompatibility; they have no specific immunogenicity in their own right, ie. they do not elicit specific antibodies. The carbohydrate core provides adequate immunopotentiation while providing a safe and naturally- derived, minimally toxic alternative to 15 established methodologies.

Furthermore, the unique fixed structural features of carbohydrates allow for multiple presentation of a pharmacologically interesting molecule in a three- dimensional space. This provides the opportunity for a more 20 concerted approach to antigen delivery, whereby optimal distances between antigenic epitopes can be established for the highest activity, even to the extent of employing two different epitopes in concert. For instance it may be advantageous to provide a molecule where improved 25 immunoprophylaxis is achieved by raising antibodies to more than one epitope.

The compounds, compositions and methods of the invention are useful for immunoprophylaxis, toxin binding or drug delivery. In particular the compound of formula I 30 is useful as a deliver system for pharmaceutically-active agents.

The term "subject" as used herein refers to any animal having a disease or condition which requires treatment with a pharmaceutically-active agent. The subject 35 may be a human, or may be a domestic or companion animal. While it is particularly contemplated that the compounds of the invention are suitable for use in medical treatment of

humans, it is also applicable to veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, cattle and sheep, or zoo animals such as felids, canids, bovids, and 5 ungulates.

The methods of this invention involve in one embodiment, (1) the administration of compound of formula I, prior to, together with, or subsequent to the administration of a pharmaceutically-active agent; or (2) 10 the administration of a combination of compound of formula I and a pharmaceutically-active agent.

As used herein, the term "therapeutically effective amount" is meant an amount of a compound of the present invention effective to yield a desired therapeutic 15 response. For example to prevent or treat a disease which is effected by administration of a pharmaceutically-active agent.

The specific "therapeutically effective amount" will, obviously, vary with such factors as the particular 20 condition being treated, the physical condition of the subject, the type of animal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compound or its derivatives.

25 As used herein, a "pharmaceutical carrier" is a pharmaceutically acceptable solvent, suspending agent or vehicle for delivering the compound of formula I and/or pharmaceutically-active agent to the subject. The carrier may be liquid or solid and is selected with the planned 30 manner of administration in mind.

The compound of formula I and/or pharmaceutically-active agent may be administered orally, 35 topically, or parenterally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes subcutaneous injections,

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aerosol, intravenous, intramuscular, intrathecal, intracranial, injection or infusion techniques.

The present invention also provides suitable topical, oral, and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compounds of the present invention may be administered orally as tablets, aqueous or oily suspensions, lozenges, troches, powders, granules, emulsions, capsules, syrups or elixirs. The composition for oral use may contain one or more agents selected from the group of sweetening agents, flavouring agents, colouring agents and preserving agents in order to produce pharmaceutically elegant and palatable preparations. The tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets.

These excipients may be, for example, (1) inert diluents, such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents, such as corn starch or alginic acid; (3) binding agents, such as starch, gelatin or acacia; and (4) lubricating agents, such as magnesium stearate, stearic acid or talc. These tablets may be uncoated or coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. Coating may also be performed using techniques described in the U. S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

The compound of formula I as well as the pharmaceutically-active agent useful in the method of the invention can be administered, for in vivo application, parenterally by injection or by gradual perfusion over time independently or together. Administration may be intravenously, intra-arterial, intraperitoneally,

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intramuscularly, subcutaneously, intracavity, or transdermally. For *in vitro* studies the agents may be added or dissolved in an appropriate biologically acceptable buffer and added to a cell or tissue.

5 Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic
10 esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated
15 Ringer's intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, anti-microbials, anti-oxidants, chelating agents, growth factors and inert gases and the like.

It is envisioned that the invention can be used to prevent or treat any disease associated with the use of pharmaceutically-active agents, including, for example, neoplasms, cancers (eg., cancers of the breast, lung, prostate, kidney, skin, neural, ovary, uterus, liver, pancreas, epithelial, gastric, intestinal, exocrine, endocrine, lymphatic, haematopoietic system or head and neck tissue), fibrotic disorders and the like. Also, disorders of the central nervous system including
25 Alzheimer's disease (AD) and other forms of dementia and memory loss, motor neurone diseases, disorders of cardiovascular system including cardiac hypertrophy, congestive heart failure, hypertension, hormonal imbalance, atherosclerosis, disorders of development and growth
30 including, disorders of glucose and fat metabolism and the like.

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Generally, the terms "treating", "treatment" and the like are used herein to mean affecting a subject, tissue or cell to obtain a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in 5 terms of completely or partially preventing a disease or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure of a disease. "Treating" as used herein covers any treatment of, or prevention of disease in a vertebrate, a mammal, particularly a human, 10 and includes: (a) preventing the disease from occurring in a subject that may be predisposed to the disease, but has not yet been diagnosed as having it; (b) inhibiting the disease, ie., arresting its development; or (c) relieving or ameliorating the effects of the disease, ie., cause 15 regression of the effects of the disease.

The invention includes various pharmaceutical compositions useful for ameliorating disease. The pharmaceutical compositions according to one embodiment of the invention are prepared by bringing a compound of 20 formula I, analogue, derivatives or salts thereof and one or more pharmaceutically-active agents or combinations of compound of formula I and one or more c pharmaceutically-active agents into a form suitable for administration to a subject using carriers, excipients and additives or 25 auxiliaries. Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as 30 sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous 35 solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in Remington's Pharmaceutical Sciences, 15th ed.

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Easton: Mack Publishing Co., 1405-1412, 1461-1487 (1975) and The National Formulary XIV., 14th ed. Washington: American Pharmaceutical Association (1975), the contents of which are hereby incorporated by reference. The pH and exact 5 concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See Goodman and Gilman's The Pharmacological Basis for Therapeutics (7th ed.).

The pharmaceutical compositions are preferably 10 prepared and administered in dose units. Solid dose units are tablets, capsules and suppositories. For treatment of a subject, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the subject, different daily doses can 15 be used. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple 20 administration of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention may be administered locally or systemically in a therapeutically effective dose. Amounts effective for this use will, of course, depend on the severity of the disease 25 and the weight and general state of the subject.

Typically, dosages used *in vitro* may provide useful 30 guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of the cytotoxic side effects. Various considerations are described, eg., in Langer, *Science*, 249: 1527, (1990). Formulations for oral use may be in the form of hard 35 gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active

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ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions normally contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspension. Such excipients may be (1) suspending agent such as sodium carboxymethyl cellulose, methyl cellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; (2) dispersing or wetting agents which may be (a) naturally occurring phosphatide such as lecithin; (b) a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate; (c) a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadecaethylenoxycetanol; (d) a condensation product of ethylene oxide with a partial ester derived from a fatty acid and hexitol such as polyoxyethylene sorbitol monooleate, or (e) a condensation product of ethylene oxide with a partial ester derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

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Compounds of formula I may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a 5 variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

Dosage levels of the compound of formula I of the present invention are of the order of about 0.5mg to about 20mg per kilogram body weight, with a preferred dosage 10 range between about 0.5mg to about 10mg per kilogram body weight per day (from about 0.5gms to about 3gms per patient per day). The amount of active ingredient that may be combined with the carrier materials to produce a single dosage will vary depending upon the host treated and the 15 particular mode of administration. For example, a formulation intended for oral administration to humans may contain about 5mg to 1g of an active compound with an appropriate and convenient amount of carrier material which may vary from about 5 to 95 percent of the total 20 composition. Dosage unit forms will generally contain between from about 5mg to 500mg of active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific 25 compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

In addition, some of the compounds of the instant 30 invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of the invention.

The compounds of the present invention may 35 additionally be combined with other compounds to provide an operative combination. It is intended to include any chemically compatible combination of pharmaceutically-active agents, as long as the combination does not

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eliminate the activity of the compound of formula I of this invention.

The invention will now be further described by way of reference only to the following non-limiting examples. It should be understood, however, that the examples following are illustrative only, and should not be taken in any way as a restriction on the generality of the invention described above. Furthermore, it will be clearly understood that although the invention is illustrated particularly with reference to delivery of antigens, the invention is not restricted solely to the delivery of antigens, but may be employed in the delivery of any pharmaceutically active moiety. The lipidic anchor may be absent or present; when the anchor is absent, the carbohydrate scaffold becomes a useful tool for the multiple presentation of pharmaceutically active molecules of interest, displaying the unique structural features described above which allow for a fixed presentation of multiple copies of a pharmaceutically active moiety in three-dimensional space. This may be of particular benefit where multivalency plays an important binding.

Where the lipoophilic anchor is present, it may be used to target particular cell membranes, to form liposomes or to induce aggregation, by exploiting the particulate forming properties of the anchor [10,11]. The amphipathic structure of molecules comprising a drug/core structure conjugate, coupled with a lipidic anchor moiety, gives rise to characteristic aggregation behaviour. Thus the invention provides a novel vesicular drug delivery system in which both drug and particulate carrier are present in the same molecule.

The invention will now be described in detail by way of reference only to the following non-limiting examples and drawings.

ABBREVIATIONS

	AIBN	2,2'-azobisisobutyronitrile
	KLH	Keyhole limpet hemagglutinin
5	9-BBN	9-borabicyclo[3.3.1]nonane
	BSA	Bovine Serum Albumin
	FMDV	Foot and Mouth Disease Virus
	Boc	t-Butoxycarbonyl
	FAB	fast atom bombardment
10	DCC	dicyclohexylcarbodiimide
	DMF	N,N-dimethylformamide
	TFA	trifluoroacetic acid
	SDS	sodium dodecyl sulfate
	Ac ₂ O	acetic anhydride
15	Pth	phthalimide
	DEAD	diethyl azodicarboxylate
	EtOAc	ethyl acetate
	THF	Tetrahydrofuran
	MeOH	methanol
20	THP	tetrahydropyran
	PTSA	p-toluenesulfonic acid
	Fmoc	9-Fluorenylmethoxycarbonyl
	HOBT	Hydroxybenzotriazole
	DIPEA	diisopropylethylamine
25	HBTU	O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate
	TIS	tri-isopropylsilane

General Methods

30 Synthesis, Purification and Characterisation of peptides. The synthesis of peptides was accomplished manually or automatically by a stepwise solid phase procedure (Merrifield, 1963) on NovaBiochem *p*-MBHA resin (substitution 0.48-0.59 mmol/g resin) or Boc-Gly-Pam resin (substitution 0.83 mmol/g resin) and their primary structure verified by MS. Lipoamino acid and carbohydrate moieties were coupled to the resin-peptide manually. The

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synthesis of the first and every subsequent level of the peptide construction were achieved using 3-fold excess of activated (as active ester) amino acid. The protected amino acids, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium 5 hexafluorophosphate (HBTU) activating reagent and the auxiliary nucleophile hydroxybenzotriazole (HOBT) were used in equimolar quantities. N,N-diisopropylethylamine (DIEA) was used to both neutralise the resin for coupling and for in situ activation of the amino acids. When necessary, a 10 second coupling was performed, using a symmetrical anhydride of the N^α-Boc amino acids in dichloromethane (20ml) and N-methylpyrrolidinone (5ml). The protecting groups used for the synthesis of peptides were Boc groups for the α-amino-termini, Acm for Cys, 2-Cl-Z for Lys, Bzl 15 for Thr and 2-Br-Z for Tyr. In all the couplings the coupling efficiency was more than 99.8 % as indicated by quantitative ninhydrin testing. After the second coupling, deprotection of the N-terminus was performed using TFA (10ml, 2 x 1 min). The deprotected resin-peptide was 20 neutralised with 10% N,N-diisopropylethylamine in dichloromethane. The resin-peptide was carefully washed with DMF after each deprotection and neutralisation step.

Conjugation of lipoamino acids. Lipoamino acids were incorporated into the matrix core (S) - linker (X) 25 chain by as N- Boc protected residues using standard HBTU/HOBt/DIEA mediated coupling strategies. When lipoamino acids were incorporated at the C-terminus of the linker a spacer (Gly and/or GABA) was used to overcome the difficulty in attaching a lipoamino acid to resin directly. 30 -The 2-(tert-butoxycarbonylamino)-alkanoic acids were synthesized from 1-bromoderivatives (Gibbons et al., 1990). The linker-conjugate was removed from the resin support with high HF method (1.5ml p-cresol, 15 ml HF) to yield the crude construct, which was precipitated with ether and 35 redissolved in 95 % acetic acid.

Purification of peptides. Analytical HPLC separation was carried out on a Vydac C₁₈ 5 RAC column (4.6

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x 250 mm at a constant flow rate of 1.2 ml min⁻¹. Mobile phases employed were: (A) 0.06 % TFA_(aq) and (B) 0.06 % TFA in 90 % acetonitrile_(aq). HPLC grade acetonitrile (Aldrich) and water were filtered through a 23μm membrane filter and 5 degassed with helium flow prior to use. Analytical PR-HPLC was performed using a Waters 616/600S twin pump system and 486 tunable absorbance detector controlled via a Millennium software package. Post-column eluent was monitored by UV absorbance at 214 nm. The gradient employed for separation 10 was 0 - 100 % (B) in 20 min linearly (Retention times in Table 4). For preparative separation a TSK-GEL preparative C₁₈ column was used (30 x 250 mm). The gradient employed for separation was 0 - 70 % (B) in 180 min linearly, staying at this concentration for 60 min and decreasing 15 steadily to 0% (B) for 30 min at a constant flow of 7 ml min⁻¹. The gradient was effected by two microprocessor-controlled Gilson 302 single piston pumps. Compounds were detected with a Holochrome UV-VIS detector at 230 nm. Chromatographs were recorded with an LKB 2210 single 20 channel chart recorder. Mass spectra were run on a Fisons VG-TOF spectrometer using matrix assisted laser desorption ionisation (MALDI) or a VG Analytical ZAB- SE instrument, using fast atom bombardment (FAB) ionisation or a Finnigan MassLab Navigator Quadrupole Mass Spectrometer using 25 electrospray ionisation. N₂ flow: 300 l/hr, temp. 180°C, cone voltage 49 V.

Quality control for purity and correct sequencing of the peptides was carried out by HPLC analysis and Fast gel electrophoresis. Additional criteria used were FAB MS, 30 NMR, IEF. For "large" proteins gas phase microsequencing was used to confirm the correct sequence.

Infra-red spectra were recorded with a Perkin Elmer 841 spectrophotometer. 1 H-NMR spectra were obtained on Varian ZL-300 and Bruker AM500 instruments, operating at 35 fields of 300 and 500 MHz respectively.

Production of antibodies against the peptides. To increase the immunogenicity of the peptides and meet the

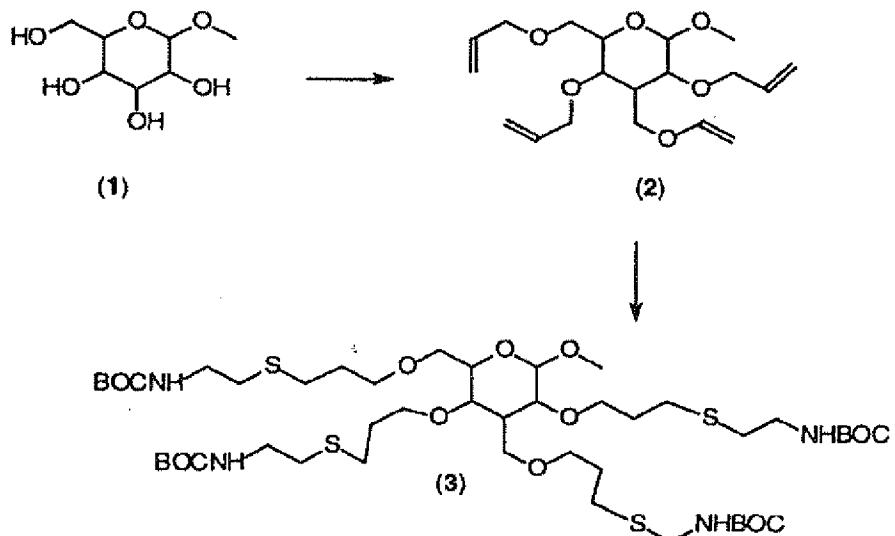
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requirement of ELISA for antibody assay, the carrier proteins is conjugated to the peptides. Either KLH or BSA may be used as the immunogen carrier in the ELISA experiments. The glutaraldehyde method is typically used 5 for conjugation, but other conjugating agents such as HOBT, water-soluble carbodiimide etc. may be used if necessary. The unused coupling agent is removed by passing through Sephadex G-25 column. Conjugated samples are lyophilized and kept at -186°C.

10 The system is injected directly. Rabbits are used to produce polyclonal antibodies using the immunization protocol 1-21-35-60, i.e. immunisation on days 1, 21 35 and 60.

15 Where necessary affinity chromatography with protein A columns or peptide-coupled Sepharose CL-4B columns is used to isolate antibody to each peptide. The purity of the antibodies is analysed by SDS-PAGE.

EXAMPLE 1 PREPARATION OF "SULFIDE" TYPE MATRIX CORE -
LINKER (S-L)



Scheme 1

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Methyl 2,3,4,6-tetra-O-allyl- α -D-glucopyranoside (2).

To a well stirred solution of **1** (3.88 g, 20.0 mmol) in dry DMF sodium hydride (60% oily dispersion, 4.00 g, 100 mmol) was added portionwise at ice bath temperature under N₂. After 30 min stirring allyl bromide (10.4 mL, 120 mmol) was added dropwise and the temperature was raised to 50°C. When the reaction was complete (about 1 hour) the mixture was placed in an ice bath and the excess of hydride was quenched by slow addition of methanol (20mL). The mixture was evaporated to dryness *in vacuo*. The residue was dissolved in CH₂Cl₂ (200 mL) and washed with water (3x80 mL). The organic phase was dried (MgSO₄), filtered, and concentrated. The residual oil was purified on silica gel by hexane-ethyl acetate (8:2) eluent yielding a colorless oil (4.77 g, 67%, R_f 0.19 hexane-EtOAc; 8:2); [α]²⁴_D +96.0 (c 1.0, CHCl₃); MS (FAB): 377 (M+Na)⁺, 355 (M+H)⁺; ¹H NMR (500 MHz, CDCl₃): δ 3.37 (s, 3H, CH₃), 3.38 (dd, 1H, J_{2,3} 9.0 Hz, H-2), 3.45 (dd, 1H, J_{3,4} 9.2 Hz, H-4), 3.62 (3H, m, H-5, H-6, H-6') 3.68 (t, 1H, J_{3,4} 9.2 Hz, H-2), 3.97-4.34 (m, 8H, 4 OCH₂CHCH₂), 4.74 (d, 1H, J_{1,2} 3.6 Hz, H-1), 5.10-5.28 (m, 8H, J_{gem} 1.2 Hz, 4 OCH₂CHCH₂), 5.87-5.93 (m, 4H, J_{vic} 5.6 Hz, 4 OCH₂CHCH₂); ¹³C NMR (62.9 Hz, CDCl₃): δ 55.0 (OCH₃), 66.7, 70.0, 72.5, 73.8, 74.2 (allyl-C-1), 77.5, 79.5, 81.5, 98.3 (C-1), 116.2, 116.5, 117.0, 117.4 (allyl-C-3), 134.7, 134.9, 135.0 (allyl-C-2). Anal. Calcd for C₁₉H₃₀O₆: C, 64.38; H, 8.53. Found: C, 64.21; H, 8.22.

Methyl 2,3,4,6-Tetra-O-((N-^tButoxycarbonylaminoethyl thio)propyl)- α -D-galactopyranoside (3).

A solution of methyl 2,3,4,6-tetra-O-allyl- α -D-glucopyranoside **2** (0.37 g, 1.04 mmol) and N-(*tert*-butoxycarbonyl)cysteamine (1.48 g, 8.35 mmol) in dioxane (20 mL) was degassed by refluxing for 10 min under an atmosphere of argon. To the cooled solution was added AIBN (10 mg) and the solution was refluxed. After 6 h the solution was diluted with diethyl ether (200 mL) and washed

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with 10% sodium hydroxide (3 x 100 ml), water (100 ml) and brine (50 ml), then dried ($MgSO_4$) and evaporated to a pale yellow oil (0.48 g). This was filtered through a short silica column and eluted with 25% diethyl ether in hexane.

5 Evaporation of the filtrate gave a pale yellow oil (0.44 g, 40%).

MS (electrospray) 1086 ($M+Na$) $^+$, 1064 (MH^+), 964 ($M-Boc$) $^+$.

R_f 0.40 diethyl ether-hexane; 1:3

10 1H n.m.r. (300 MHz, $CDCl_3$) 0.87, 8H, m, 4 x SCH_2CH_2 ; 1.40, 36H, 4 x CMe_3 ; 2.77, 8H, t (J 6.3), 4 x SCH_2 ; 2.90, 8H, t (J 6.5), 4 x SCH_2 ; 3.28-3.36, 8H, 4 x NCH_2 ; 3.38-3.45, 11H, OMe and 4 x NCH_2 ; 4.95, 2H, br s, 2 x NH ; 5.17, 2H, br s, 2 x NH .

15

Methyl 2,3,4,6-Tetra-O-(3'-(2''-aminoethylthio)propyl)- α -D-galactopyranoside (4).

20 A solution of methyl 2,3,4,6-Tetra-O-(3'-(N -Butoxycarbonyl aminoethylthio)propyl)- α -D-galactopyranoside (3) in dichloromethane is treated with trifluoroacetic acid for 5 minutes at room temperature. After this time the solvents and trifluoroacetic acid are removed in vacuo and the salt of the free amine treated with diisopropylethylamine to yield the free amine.

25

EXAMPLE 2 IMMOBILISATION OF AN ANTIGEN TO "SULFIDE"
TYPE MATRIX CORE THROUGH A UREA BOND

30 *Methyl 2,3,4,6-Tetra-O-(3'-(2''-aminoethylthio)propyl)- α -D-galactopyranoside (4)* is treated with triphosgene in dichloromethane and diisopropylethylamine to generate the isocyanate in situ. After stirring for 30 minutes at room temperature, a pharmaceutically active agent containing a free amino function is added to the solution to form a urea linkage by 35 reaction with the isocyanate.

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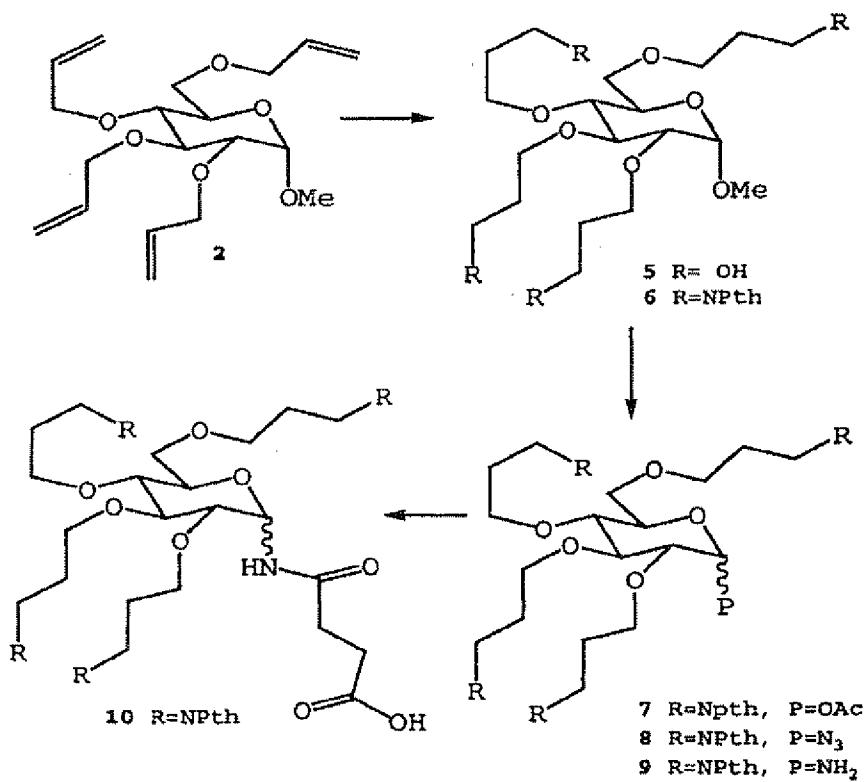
EXAMPLE 3IMMOBILISATION OF AN ANTIGEN TO "SULFIDE"
TYPE MATRIX CORE THROUGH AN AMIDE BOND

Methyl 2,3,4,6-Tetra-O-(3'-(2'-'-aminoethylthio)propyl)- α -D-galactopyranoside (4) is dissolved in DMF and to this solution is added a solution of a pharmaceutically active agent containing a free carboxylate function, HOBT and diisopropylethylamine. The mixture is stirred at room temperature for 30 minutes to form an amide linkage by reaction of the activated carboxylate with the free amine.

EXAMPLE 4PREPARATION OF AN "ALKYL" TYPE MATRIX CORE -
LINKER (S-L)

Synthesis of oligosaccharide core matrix molecules as building blocks for carbohydrate-centred macromolecules was carried out according to scheme 2 and scheme 3.

Scheme 2



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Methyl 2,3,4,6-tetra-O-(3-hydroxy-propyl)- α -D-glucopyranoside (5).

To a solution of **2** (1.03 g, 2.9 mmol) in dry THF (25 mL) 9-BBN (0.5M solution in THF; 70 mL, 35 mmol) was added under nitrogen and the reaction was stirred at reflux for 6 h. Then the excess of 9-BBN was destroyed by dropwise addition of water (3.0 mL) at 0 °C. The hydroboration mixture was oxidized by adding 3M aq NaOH (36 mL) and 30% H₂O₂ (36 mL) slowly at 0°C followed by stirring overnight at room temperature. The aqueous phase was saturated with K₂CO₃ and the THF phase was separated. The aqueous phase was extracted with THF (2x50 mL). The combined THF layers were dried over MgSO₄, filtered, and concentrated. The oily residue was purified by column chromatography (9:1→8:2 CHCl₃-MeOH) to yield a colorless oil (0.86 g, 70%; R_f 0.26 CHCl₃-MeOH; 8:2): MS(FAB): 449 (M+Na)⁺, 427 (M+H)⁺; ¹H NMR (500 MHz, CDCl₃): δ 1.77-1.82 (m, 8H, 4 OCH₂CH₂CH₂OH), 3.24 (dd, 1H, J_{4,5} 9.2 Hz, H-4), 3.28 (dd, 1H, H-2), 3.38 (s, 3H, OCH₃), 3.48 (1H, t, J_{3,4} 9.5 Hz, H-3), 3.52-3.74 (m, 16H, 4 OCH₂CH₂CH₂OH), 3.80 (m, 1H, H-6), 3.82-3.87 (m, 2H, H-5, H-6'), 4.80 (1H, d, J_{1,2} 3.5 Hz, H-1). Anal. Calcd for C₁₉H₃₈O₁₀: C, 53.51; H, 9.00. Found: C, 53.60; H, 8.72.

Methyl-2,3,4,6-tetra-O-3-phthalimidopropyl- α -D-glucopyranoside (6).

To a solution of **5** (0.48 g, 1.13 mmol), phthalimide (0.93 g, 6.30 mmol), and triphenylphosphine (1.57 g, 6.0 mmol) in dry THF (40 mL) diethyl azodicarboxylate (DEAD) (0.93 mL, 5.9 mmol) dissolved in dry THF (5 mL) was added dropwise and the reaction was stirred at room temperature under N₂ for 72 h. The solvent was evaporated *in vacuo* and the residue dissolved in CH₂Cl₂ (50 mL) was washed with brine and dried (MgSO₄), filtered and concentrated *in vacuo*. Purification of the residue with ethyl acetate - hexane (8:2) eluent afforded the product (1.0 g, 94%, R_f 0.28 EtOAc - hexane; 7:3). [α]²⁴_D +28.5 (c 1.0, CHCl₃); MS (FAB): 966 (M+Na)⁺, 943 (M)⁺; ¹H

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NMR (500 MHz, CDCl₃): δ 1.91-1.98 (m, 8H, 4 OCH₂CH₂CH₂NPh),
3.06-3.11 (m, 2H, H-4, H-2), 3.29 (s, 3H, OCH₃), 3.43 (t,
1H, J_{3,4} 9.5 Hz, H-3), 3.46-3.63 (m, 8H, 4 OCH₂CH₂CH₂NPh),
3.65-3.92 (m, 11H, 4 OCH₂CH₂CH₂NPh, H-5, H-6, H-6'), 4.70
5 (d, 1H, J_{1,2} 3.5 Hz, H-1), 7.45-7.80 (16H, m, 4 ArH); ¹³C
NMR (62.9 Hz, CDCl₃): 28.8, 29.3, 29.4, 29.6
(OCH₂CH₂CH₂NPh), 35.3, 35.7, 35.8 (OCH₂CH₂CH₂NPh), 54.9
(OCH₃), 68.7, 69.2, 69.8, 70.0, 70.6, 71.0, 76.5
(OCH₂CH₂CH₂NPh, C-5, C-6), 78.24 (C-4), 80.8 (C-2), 81.9
10 (C-3), 97.7 (C-1), 123.0, 123.1, 131.9, 132.0, 132.2,
132.4, 133.7, 133.8 (ArC), 168.2 (CONPh). Anal. Calcd for
C₅₁H₅₀O₁₄N₄: C, 64.96; H, 5.34. Found: C, 64.68; H, 5.42.

1-O-Acetyl-2,3,4,6-tetra-O-3-phthalimidopropyl- α -D-
15 glucopyranose (7).

A solution of 6 (1.0 g, 1.06 mmol) in acetic anhydride (10 mL) was stirred at -20°C for 10 min. To this stirred solution was added precooled (0°C) Ac₂O/H₂SO₄ (50:1, 5 mL) in 5 min, and the reaction mixture was left at -20°C
20 for 3 days. The reaction mixture was diluted with dichloromethane (100 mL) and was washed successively with sat. NaHCO₃ (50 mL) and water (50 mL). The organic layer was dried (MgSO₄), filtered, and concentrated in vacuo and co-distilled with toluene several times. The residue was
25 purified on silca gel column with ethyl acetate - hexane (7:3) solvent to yield a colourless oil (0.8 g, 78%; R_f 0.19); MS(FAB): 1104 (M+Cs)⁺, 994 (M+Na)⁺; ¹H NMR (500 MHz, CDCl₃): δ 1.91-1.95 (m, 8H, 4 OCH₂CH₂CH₂NPh), 2.10 (s, 3H, OAc), 3.14-3.19 (2H, m, H-4, H-2), 3.40 (m, 1H, H-3), 3.45
30 (m, 1H, H-6), 3.51-3.79 (m, 17H, H-6', 4 OCH₂CH₂CH₂NPh, 3.82-3.90 (m, 2H, H-3, H-5), 6.12 (1H, d, J_{1,2} 3.5 Hz, H-1), 7.45-7.80 (m, 16H, 4ArH); ¹³C NMR (62.9 Hz, CDCl₃): δ 21.0
(Ac-C-1) 28.8, 29.2, 29.4, 29.5 (OCH₂CH₂CH₂NPh), 35.3, 35.6
(OCH₂CH₂CH₂NPh), 68.5, 69.3, 69.3, 70.9, 71.1, 72.8, 76.5
35 (OCH₂CH₂CH₂NPh, C-5, C-6), 77.5 (C-4), 79.7 (C-2), 81.6 (C-3), 89.6 (C-1), 123.0, 123.1, 131.9, 132.0, 132.2, 132.3,

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133.7, 133.7 (ArC), 168.2 (CONPht). Anal. Calcd for C₅₂H₅₀O₁₅N₄: C, 64.32; H, 5.19. Found: C, 64.41; H, 5.22.

2,3,4,6-Tetra-O-3-phthalimidopropyl- α / β -D-glucopyranosyl 5 azide (8).

A solution of 7 (0.44 g, 0.45 mmol) in dry CH₂Cl₂ (20 mL) was stirred with azidotrimethylsilane (0.15 mL, 1.13 mmol) and tin(IV)chloride (0.026 mL, 0.23 mmol) for 1 day. The solution was diluted with dichloromethane (20 mL) 10 and washed with 1M KF solution (10 mL) then with water (10 mL). The organic extract was dried (MgSO₄), filtered, and concentrated to afford a white foam (0.36 g, 83%; R_f 0.30 EtOAc-hexane; 7:3). [α]²⁴_D +51.8 (c 1.0, CHCl₃); MS(FAB): 977 (M+Na)⁺, 955 (M+1)⁺; ¹H NMR(500 MHz, CDCl₃): 1.89-1.97 15 (m, 8H, 4 OCH₂CH₂CH₂NPht), 3.06-3.15 (m, 2H, H-2, H-4), 3.29 (t, 1H, J_{2,3} 9.0 Hz, H-3), 3.44-3.87 (m, 19H, H-5, H-6, H-6', 4 OCH₂CH₂CH₂NPht), 5.36 (1H, d, J_{1,2} 3.5 Hz, H-1), 7.45-7.80 (m, 16H, 4 ArH). Anal. Calcd for C₅₀H₄₇O₁₃N₇: C, 63.47; H, 4.97. Found: C, 63.41; H, 4.88.

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2,3,4,6-Tetra-O-3-phthalimidopropyl- α / β -D-glucopyranosylamine (9).

The azido sugar 8 (0.38 g, 0.4 mmol) dissolved in ethyl acetate (10 mL) was hydrogenated using Pd (10% on 25 charcoal, 90 mg, 10%) catalyst for 2 days at room temperature. The catalyst was filtered off and washed with ethyl acetate (40 mL) and the filtrate was evaporated. The residue was purified with ethyl acetate-ether (9:1) eluent containing 0.5% triethylamine. The product (280 mg, 76%; R_f 0.21) is a white foam; MS (FAB): 951 (M+Na)⁺, 928 (M)⁺; ¹H NMR(500 MHz, CDCl₃): δ 1.84-1.99 (m, 8H, 4 OCH₂CH₂CH₂NPht), 3.01-3.11 (m, 3H, H-4, H-2, H-3), 3.44-3.92 (m, 19H, H-5, H-6, H-6', 4 OCH₂CH₂CH₂NPht), 4.95 (t, 1H, H-1), 7.45-7.80 30 (m, 16H, 4 ArH); ¹³C NMR(62.9 Hz, CDCl₃): δ 28.7, 28.9, 29.4, 29.6 (OCH₂CH₂CH₂NPht), 35.4, 35.7 (OCH₂CH₂CH₂NPht), 69.3, 70.0, 70.2, 70.4, 70.8, 71.0, 75.6 (OCH₂CH₂CH₂NPht, C-35, C-6), 78.6 (C-4), 84.1 (C-2), 85.9 (C-3), 89.3 (C-1),

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123.1, 131.88, 132.4, 133.5, 133.6, 133.7 (ArC), 166.2 (CONPht). Anal. Calcd for C₅₀H₄₉O₁₃N₅: C, 64.72; H, 5.32. Found: C, 64.41; H, 5.12.

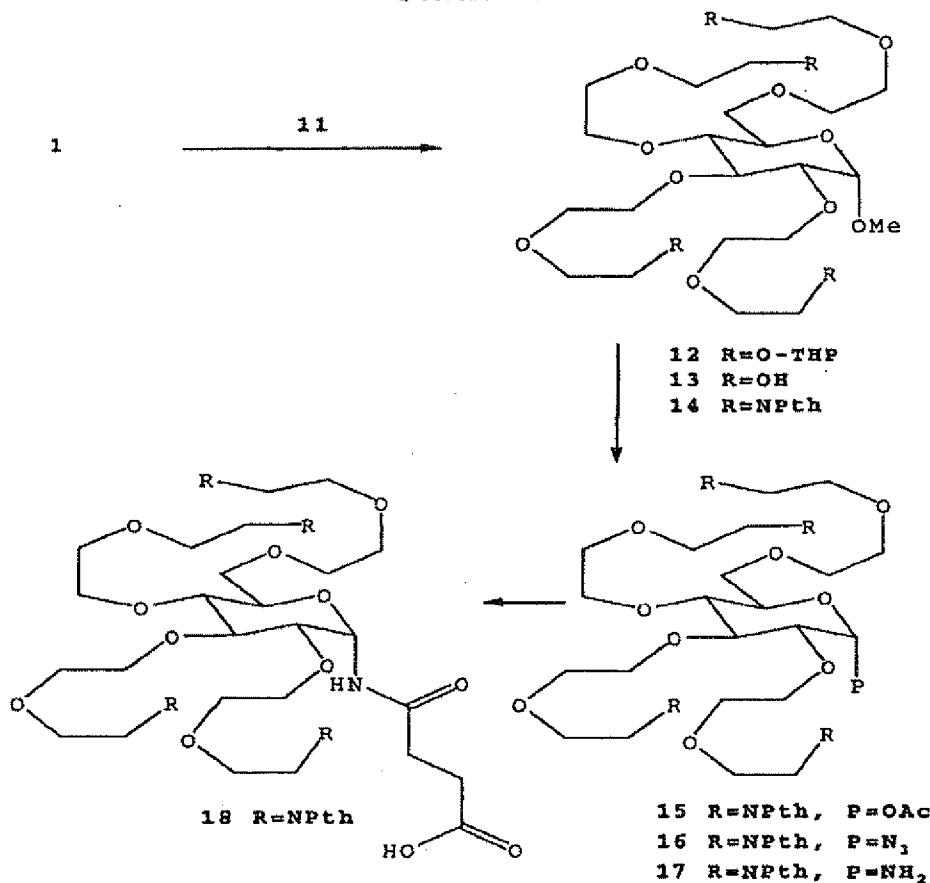
5 4-[2,3,4,6-tetra-O-3-phthalimidopropyl- α / β -D-glucopyranosylamino]-4-oxobutanoic acid (10).

The amino sugar 9 (140 mg, 0.15 mmol) was refluxed with succinic anhydride (17 mg, 1.7 mmol) in dry CH₂Cl₂ (5 mL) overnight. The solvent was evaporated and the residue was purified by chromatography with CHCl₃-MeOH (93:7). The product (130 mg, 84%, R_f 0.33 CHCl₃-MeOH; 9:1) is a white foam; [α]²⁴_D +24.0 (c 1.0, CHCl₃); MS(FAB): 1051 (M+Na)⁺, 1029 (M+1)⁺; ¹H NMR (500 MHz, CDCl₃): δ 1.79-1.96 (m, 8H, 4 OCH₂CH₂CH₂NPht), 2.59 (m, 2H, HNOCCH₂), 2.73 (m, 15 2H, HOOCCH₂), 2.96 (m, 1H, H-2), 3.19 (m, 2H, H-5, H-3), 3.32 (m, 1H, H-6), 3.41 (m, 1H, H-6'), 3.46-3.83 (m, 17H, H-4, 4 OCH₂CH₂CH₂NPht), 4.86 (t, 1H, J_{1,2} 8.5Hz, H-1), 7.47 (d, 1H, NH), 7.62-7.80 (m, 16H, 4 ArH); ¹³C NMR (62.9 Hz, CDCl₃): 28.8, 29.0, 29.4, 29.6 (OCH₂CH₂CH₂NPht), 30.7 (NCOCH₂) 34.9, 35.6 (OCH₂CH₂CH₂NPht), 69.0, 69.2, 69.3, 69.6, 70.3, 70.5, 70.8, 76.3 (OCH₂CH₂CH₂NPht, C-5, C-6), 79.0 (C-4), 78.2, 80.9 (C-2), 81.5 (C-3), 86.3 (C-1), 123.1, 123.2, 123.3, 123.4, 132.0, 132.1, 132.2, 133.8, 133.9, 134.0, 134.1 (ArC), 166.2, 166.37, 166.9 (CONPht), 25 173.2 (NHCO). Anal. Calcd for C₅₄H₅₃O₁₆N₅: C, 63.09; H, 5.20; N, 6.81. Found: C, 63.21; H, 5.31, N, 6.64.

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EXAMPLE 5: PREPARATION OF AN "ETHER" TYPE MATRIX CORE -
LINKER (S-L)

Scheme 3



5

2-[2-(2-iodoethoxy)ethoxy]tetrahydro-2H-pyran (11).

2-Chloroethoxyethanol (6.0 g, 48 mmol) was added to ice-cold 3,4-dihydropyran (15 mL) under anhydrous conditions and the mixture was stirred for 5 h at room temperature. The excess of unreacted dihydropyran was distilled off in motor vacuum. The pale yellow oily residue (9.8 g, R_f 0.41 hexane-ethyl acetate; 1:1) was added without further purification to a solution of dry sodium iodide (8.8 g, 58.8 mmol) in dry acetone (60 mL) at room temperature, and the mixture was stirred for 24 hours under reflux. The precipitated salt was filtered off and the

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solvent was evaporated. The residue was taken up with dichloromethane (60 mL) and the precipitating salt was filtered off again. The filtrate was washed with 40 mL of water. The organic phase dried ($MgSO_4$) and concentrated.

5 The oily product (13.5 g, 82%, R_f 0.30 hexane-ethyl acetate; 8:2) was used in the next step without purification; MS(FAB): 215 ($M+Na$)⁺; 1H NMR (500 MHz, $CDCl_3$): δ 1.51-1.83 (m, 6H), 3.37 (t, 2H), 3.50 (m, 1H), 3.61 (m, 1H), 3.69 (m, 5H), 3.87 (m, 1H), 4.62 (m, 1H, OCHO), ^{13}C NMR (62.9 Hz, $CDCl_3$): 19.4, 25.6, 30.5, 50.7, 62.2, 66.7, 70.0, 70.6, 74.5, 99.0.

Methyl 2,3,4,6-tetra-O-1-[2-(tetrahydro-2H-pyranyl)oxy]-3-oxapentyl- α -D-glucopyranoside (12).

15 Sodium hydride (60% oily dispersion, 8.0 g, 200 mmol) was added to the stirred solution of methyl α -D-glucoside (4.85 g, 25 mmol) in anhydrous DMF (125 mL) at ice-bath temperature under nitrogen. After stirring for 30 min 2-[2-(2-iodoethoxy)ethoxy]tetrahydro-2H-pyran 11 was 20 added dropwise at 0°C. The cooling bath was then removed and the flask was immersed into an oil bath at 50°C. After 16 h the reaction mixture was concentrated by rotary evaporation. The residue was dissolved in CH_2Cl_2 (300 mL) and washed with water (2x100 mL). The organic phase was 25 dried ($MgSO_4$), filtered, and concentrated. The oily residue was purified on silica gel with $CHCl_3 \rightarrow CHCl_3$ -MeOH (95:5) eluents to afford the yellow oily product (4.2 g, 19%, R_f 0.37 $CHCl_3$ -MeOH; 95:5); MS(FAB): 906 ($M+Na$)⁺; 1H NMR (500 MHz, $CDCl_3$): δ 1.54 (m, 16H, CH_2 of THP), 1.68 (m, 4H, CH_2 of THP), 1.79 (m, 4H, CH_2 of THP), 3.27 (m, 1H, H-2), 3.34 (s, 3H, CH_3), 3.42-3.85 (m, 4H), 3.51-3.76 (m, 29H), 3.78-3.85 (m, 9H), 3.90-4.07 (m, 3H), 4.59 (4H, s, CH of THP), 4.76 (1H, d, $J=3.5$ Hz, H-1). Anal. Calcd for $C_{43}H_{78}O_{18}$: C, 58.49; H, 8.90. Found: C, 58.11; H, 8.99.

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Methyl 2,3,4,6-tetra-O-(1-hydroxy-3-oxapentyl)- α -D-glucopyranoside (13).

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To a solution of **11** (4.2 g, 4.8 mmol) in CHCl₃ (30 mL) and MeOH (30 mL) p-toluene-sulfonic acid monohydrate (0.23 g, 1.2 mmol) was added. The solution was stirred at 55°C for 2 h. The catalyst was neutralized by 5 the addition of triethylamine (0.2 mL) and the solvent was evaporated. The residue was purified on silica gel with CHCl₃-MeOH (9:1) eluent. The product (1.8 g, 69%; R_f 0.19 CHCl₃-MeOH; 85:15) is a colorless oil; MS(FAB): 679 (M+Cs)⁺, 585 (M+K)⁺, 569 (M+Na)⁺; ¹H NMR(500MHz, CDCl₃): δ 10 3.17 (t, 1H, J_{2,3} 9.5Hz, H-2), 3.26 (dd, 1H, J_{5,6} 6.5 Hz, H-5), 3.31 (s, 3H, OCH₃), 3.42-3.3.61 (m, 29H), 3.60 (m, 2H), 3.66 (m, 2H), 3.70 (m, 1H), 3.87 (m, 2H), 4.79 (d, 1H, J=3.4 Hz, H-1). Anal. Calcd for C₂₃H₄₆O₁₄: C, 50.54; H, 8.48. Found: C, 50.41; H, 8.21.

15 *Methyl 2,3,4,6-tetra-O-(1-phthalimido-3-oxapentyl)-α-D-glucopyranoside (14).*

To a solution of **12** (1.8 g, 3.3 mmol), phthalimide (2.72 g, 18.5 mmol), and triphenylphosphine 20 (4.58 g, 17.5 mmol) in dry THF (15 mL) diethyl azodicarboxylate (DEAD) (2.69 mL, 17.1 mmol) dissolved in dry THF (5 mL) was added dropwise and the reaction was stirred at room temperature under nitrogen for 72 h. The solvent was evaporated in vacuo and the residue, dissolved 25 in CH₂Cl₂ (100 mL), was washed with brine and dried (MgSO₄), filtered, and concentrated. Purification of the residue on silca gel column with ethyl acetate - ether (9:1) eluent afforded the product (3.0 g, 86%; R_f 0.22); [α]²⁴_D +60.4 (c 1.0, CHCl₃); MS(FAB): 1085(M+Na)⁺; ¹H NMR(500 MHz, CDCl₃): δ 30 3.16 (m, 2H, H-2, H-5), 3.27 (s, 3H, OCH₃), 3.42-3.48 (m, 2H, H-6, 6'), 3.51-3.63 (m, 14H), 3.62-3.77 (m, 10H), 3.80-3.89 (m, 10H), 4.65 (1H, d, J=3.5 Hz, H-1), 7.44-7.83 (16H, m, arH); ¹³C NMR (62.9 Hz, CDCl₃): δ 37.3, 37.4 (CH₂N), 54.7 (OCH₃), 67.5, 67.6, 67.8, 69.5, 69.9, 70.0, 70.2, 70.4, 35 70.5, 70.8, 72.0, 72.2, 76.5, 77.8, 80.7, 82.1, 98.0, 123.2, 131.9, 132.0, 132.1, 132.2, 133.8, 168.1. Anal.

Calcd for $C_{55}H_{58}O_{18}N_4$: C, 62.14; H, 5.50. Found: C, 62.31; H, 5.32.

5 *1-O-Acetyl-2,3,4,6-tetra-O-(1-phthalimido-3-oxapentyl)- α -D-glucopyranose (15).*

A solution of **14** (3.5 g, 3.3 mmol) in acetic anhydride (15 mL) was stirred at -20°C for 10 min. To this stirred solution was added precooled (0°C) Ac_2O/H_2SO_4 (50:1, 7.5 mL) in 5 min, and the reaction mixture was left at -10 20°C for 3 days. The reaction mixture was diluted with CH_2Cl_2 (300 mL) and was washed successively with cold sat. $NaHCO_3$ (100 mL) and water (2x100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in *vacuo* and co-distilled with toluene several times. The residue was 15 purified on a silca gel column with ethyl acetate - ether (85:15) eluent to give an oily product (2.8 g, 78%; R_f 0.34 $EtOAc$ -ether; 9:1); $[\alpha]^{24}_D +33.0$ (c 1.0, $CHCl_3$); MS(FAB): 1224 ($M+Cs$) $^+$, 1114 ($M+Na$) $^+$; 1H NMR (500 MHz, $CDCl_3$): δ 2.02 (s, 3H, CH_3 of OAc), 3.20-3.28 (m, 2H, H-2, H-5), 3.52-3.75 (m, 24H, CH_2 and sugar protons), 3.72-3.91 (m, 12H, CH_2 and sugar protons), 6.14 (1H, d, $J_{1,2}$ 3.5 Hz, H-1), 7.63-7.82 (16H, m, arH); ^{13}C NMR (62.9 Hz, $CDCl_3$): δ 21.0, 37.3, 37.4, 67.6, 67.7, 67.8, 67.9, 69.2, 69.9, 70.2, 70.4, 70.6, 70.7, 70.8, 72.3, 72.7, 76.5, 78.8, 81.7, 90.0, 123.2, 132.2, 25 133.9, 168.6. Anal. Calcd for $C_{56}H_{58}O_{19}N_4$: C, 61.65; H, 5.36. Found: C, 61.27; H, 5.23.

2,3,4,6-Tetra-O-(1-phthalimido-3-oxapentyl)- α/β -D-glucopyranosyl azide (16).

30 A solution of **15** (1.3 g, 1.2 mmol) in dry CH_2Cl_2 (30 mL) was stirred with azidotrimethylsilane (0.4 mL, 3.0 mmol) and tin (IV)chloride (0.07 mL, 0.6 mmol) for 24 h at room temperature. The solution was diluted with an equal volume of CH_2Cl_2 and washed with 1M KF solution (30 mL) 35 then with water (50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated in *vacuo* to afford a white foam (1.13g, 88%; R_f 0.41 $EtOAc$ -ether; 9:1); $[\alpha]^{24}_D$

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+18.6 (c 1.0, CHCl_3); MS(FAB): 1207 (90, $\text{M}+\text{Cs}^+$), 1097 (39, $\text{M}+\text{Na}^+$); ^1H NMR (500 MHz, CDCl_3): δ 3.25 (2H, m, H-2, H-5), 3.47-3.69 (24H, m, CH_2 and sugar protons), 3.70-3.88 (12H, m, CH_2 and sugar protons), 5.94 (1H, d, $J=3.5$ Hz, H-1), 7.54-7.80 (16H, m, arH); ^{13}C NMR (62.9 Hz, CDCl_3): δ 37.4, 67.9, 68.0, 69.2, 69.5, 70.0, 70.3, 70.5, 70.7, 71.00, 71.4, 72.0, 72.3, 72.5, 76.5, 77.5, 80.8, 81.9, 85.0, 87.8, 91.0, 123.2, 132.2, 133.7, 166.1. Anal. Calcd for $\text{C}_{54}\text{H}_{55}\text{O}_{17}\text{N}_7$: C, 60.39; H, 5.16. Found: C, 60.52; H, 5.01.

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2,3,4,6-Tetra-O-(1-phthalimido-3-oxapentyl)- α/β -D-glucopyranosylamine (17).

The azido sugar 16 (110 mg, 0.1 mmol) dissolved in ethyl acetate (10 mL) was hydrogenated using Pd catalyst (10% on charcoal, 60 mg) for 2 days at room temperature. The catalyst was filtered off and washed with ethyl acetate (40 mL) and the filtrate was evaporated. The residue was purified on silica gel with chloroform-methanol (97:3). The product (80 mg, 76%; R_f 0.2 CHCl_3 -MeOH; 95:5) is a white foam; MS(FAB): 1071 ($\text{M}+\text{Na}^+$); ^1H NMR (500MHz, CDCl_3): δ 3.12-3.21 (m, 2H, H-2, H-5), 3.47-3.93 (m, 38H), 5.21 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 7.68-7.81 (m, 16H, 4 ArH); ^{13}C NMR (62.9 Hz, CDCl_3): δ 37.4, 67.6, 67.8, 69.7, 70.0, 70.2, 70.6, 71.7, 71.9, 72.1, 72.2, 75.5, 75.7, 76.5, 77.0, 77.9, 79.6, 81.9, 82.3, 83.9, 85.7, 86.0, 86.4, 89.1, 91.0, 123.2, 132.2, 133.9, 168.1. Anal. Calcd for $\text{C}_{54}\text{H}_{57}\text{O}_{17}\text{N}_5$: C, 61.68; H, 5.48. Found: C, 60.31; H, 5.29.

4-[2,3,4,6-Tetra-O-(1-phthalimido-3-oxapentyl)- α/β -D-glucopyranosylamino]-4-oxobutanoic acid (18).

The amino sugar 17 (45 mg, 0.04 mmol) was refluxed with the succinic anhydride (5.4 mg, 0.05 mmol) in dry CH_2Cl_2 (5 mL) overnight. The solvent was evaporated and the residue was purified by chromatography with CHCl_3 -MeOH (95:5). The product (47 mg, 84%; R_f 0.12 CHCl_3 -MeOH; 95:5) is a white foam; MS(FAB): 1171 ($\text{M}+\text{Na}^+$), 1149 ($\text{M}+1$); ^1H NMR (500MHz, CDCl_3): δ 2.59 (m, 2H, HNOCCCH_2), 2.73 (m, 2H,

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HOOCCH₂), 3.10-3.18 (m, 2H, H-2, H-5), 3.44-3.90 (m, 37H), 5.20 (d, 1H, J_{1,2} 3.5 Hz, H-1), 7.67-7.80 (m, 16H, 4 ArH); ¹³C NMR (62.9 Hz, CDCl₃): δ 30.8, 31.2, 37.4, 67.6, 67.8, 69.7, 70.0, 70.2, 70.6, 71.7, 71.9, 72.1, 72.2, 75.5, 75.7, 5 76.5, 77.0, 77.9, 79.6, 81.9, 82.3, 83.9, 85.7, 86.0, 86.4, 89.1, 91.0, 123.2, 132.2, 133.9, 168.1. Anal. Calcd for C₅₈H₆₁O₂₀N₅: C, 60.68; H, 5.35. Found: C, 60.41; H, 5.22.

Protocol for loading 10 and 18 on pre-loaded Wang resin.

10 Fmoc-Gly-Wang resin (0.1 g, 0.069 mmol) was swollen in DMF in a solid phase peptide synthesis reaction vessel for 3 h. The Fmoc protection was removed with 20% piperidine in DMF (10 mL) by shaking the resin for 2x10 min. The resin was thoroughly washed with DMF several 15 times, then 1.5 molar equivalent (0.1 mmol) of 10 or 18 was added together with 2.0 equivalents of HBTU (52 mg, 0.138 mmol) and HOBT (21 mg, 0.138 mmol) and 4.0 equivalents of DIPEA (48 μL, 0.28 mmol) coupling reagents in DMF. The vessel was shaken for 1 h then the process repeated with 20 the same amount of 10 or 18, and coupling reagents until the ninhydrin test was negative. Finally, the resin was washed with DMF several times.

25 *Protocol for phthaloyl deprotection of 10 and 18 on solid phase.*

Resin-bound glycosides 10 and 18 were treated with hydrazine hydrate (67 μL, 1.38 mmol) in ethanol (5 mL) at 60°C for 1 day. In order to check the efficiency of the deprotection, a subsample of the resin-bound glycoside was 30 thoroughly washed with DMF then with a mixture of CH₂Cl₂ - MeOH and finally with CH₂Cl₂ and dried. The glycoside was cleaved from the resin by stirring the resin with TFA-water-TIS (95/2.5/2.5) for 2 h. The resin was filtered off and washed with TFA. The solvent was evaporated and the 35 residue was neutralized, and triturated with ether. The precipitate was analyzed by MS (FAB) and elemental analysis. Cleavage of resin bound, deprotected 10 yielded

- 42 -

19, while cleavage of resin bound, deprotected 18 yielded 20.

MS (FAB); 19: 587 (M+Na)⁺, 565 (M+1)⁺; 20: 707 (M+Na)⁺, 685 (M+1)⁺.

5 Anal. Calcd for C₂₄H₄₈O₉N₆ (19): C, 51.05; H, 8.56; N, 14.88. Found: C, 50.78; H, 8.22, N, 14.50. Calcd for C₂₈H₅₆O₁₃N₆ (20): C, 49.11; H, 8.24; N, 12.27. Found: C, 49.41; H, 8.07; N, 12.36.

10 EXAMPLE 6 PREPARATION OF "ETHER" TYPE MATRIX CORE -
LINKER (S-L) DONOR SUGAR

In a cognate manner methyl thiogalactoside is used in place of 1 for the reactions described in scheme 3 to yield the thiomethyl equivalent of compound 13.

15 Thus S-Me-galactose (200 mg) was added in one portion to sodium hydride (308 mg 60% in oil dispersion) suspended in dry DMF (15 ml) at ice bath temperature under nitrogen. The mixture was stirred for 30 min and the iodoethoxyethanol-tetrahydropyranyl ether (2 equivalent/OH, 2.32 g) dissolved in dry DMF (5 ml) was added in 20 min and the resulting mixture was stirred for 2 hours at room temperature. The excess NaH was quenched with methanol and the solvent distilled off in motor vacuum. The residue (1.4 g) was dissolved in dichloromethane and washed with water.

20 The organic layer was dried over magnesium sulfate and was concentrated. The residue was purified by column chromatography using chloroform - methanol eluants (99/1 and 95/5). The product 21 (70 mg, 8.2%) is a yellow oil (R_f = 0.39 in chloroform-methanol 95/5).

25

30 FAB-MS: 945 (100, M)
¹H-NMR: 1.58-1.82 (m, 24H), 2.1 (s, 3H), 3.72-3.96 (m, 46H), 4.3 (d, 1H), 4.7 (t, 4H)
¹³C-NMR: 12.7, 19.5, 23.4, 25.5, 26.4, 61.8, 62.2, 66.7, 66.3, 68.3, 70.5, 70.7, 70.9, 72.4, 84.6, 85.5, 35 99.0.

Tetra(tetrahydropyranoyloxy-ethoxy)ethyl 1-S-methyl galactoside 21 (60 mg) dissolved in chloroform -

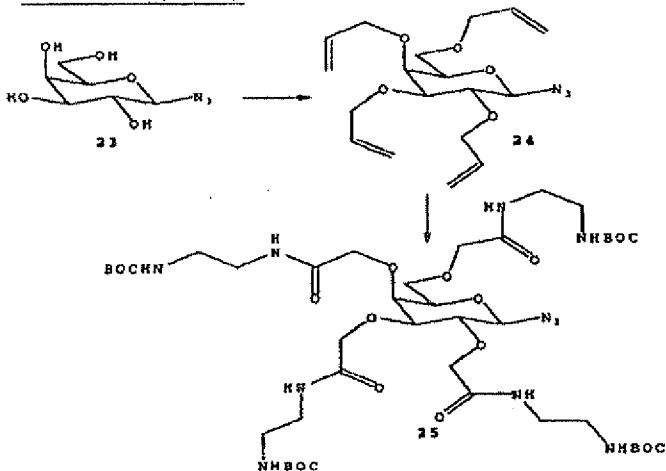
- 43 -

methanol (1/1; 20 ml) was treated with 3N HCl (30 μ l) at 50°C for 4 hours. The solvent was evaporated and the product was dried by repeated evaporation with toluene. The product 22 (36 mg, 97%) is a thick syrup.

5 FAB-MS: 586 (100, M)

10 1 H-NMR: 2.1 (s, 3H), 3.72-3.96 (m, 38H), 4.3 (d, 1H).

EXAMPLE 7: PREPARATION OF "AMIDO" TYPE MATRIX CORE -
LINKER (S-L)



Scheme 4

2,3,4,6-Tetra-O-allyl- β -D-galactopyranosyl azide 24

15 50% Aqueous sodium hydroxide (5.0 ml) was added to a stirred solution of β -D-galactosyl azide 23 [Szarek, W.A. et al., *Tetrahedron*, 1978, 34, 1427-1433] (2.99 g, 14.6 mmol) in DMSO (50 ml). Allyl bromide (7.4 ml, 10.6 g, 87 mmol) was then added and the mixture was vigorously stirred overnight, giving a clear yellow solution. This was diluted with water (200 ml) and extracted with diethyl ether (3 x 200 ml). The combined extracts were washed with water (3 x 100 ml) and brine (50 ml) then dried ($MgSO_4$) and evaporated to a yellow oil which was chromatographed on silica gel and eluted with 20% diethyl ether in hexane to give a colorless oil (1.33 g, 25%).

- 44 -

R_f 0.64 diethyl ether-hexane; 1:1
¹H n.m.r. (300 MHz, CDCl₃) 5.83-6.01, 4H, m, 4xCH₂=CH; 5.13-5.33, 8H, m, 4xCH₂=CH; 4.50, 1H, d(J 8.7), H1; 4.34, 1H, dd(J 5.5, 12.7), H6_A; 4.27, 2H, d(J 5.8), 5 OCH₂; 4.15, 2H, br d(J 5.5), OCH₂; 4.11, 1H, dd(J 6.2, 12.7), H6_B; 4.00, 4H, br t(J 5.1), 2x OCH₂; 3.79, 1H, d(J 2.9), H4; 3.61, 3H, s, OMe; 3.60, 1H, t(J 9.5), H5; 3.50, 1H, t(J 8.9), H2; 3.34, 1H, dd(J 2.8, 9.5), H3.
¹³C n.m.r. (75 MHz, CDCl₃) 68.0, 71.5, 72.3, 72.8, 10 73.7, 74.1, 75.3, 78.2, 81.8, 90.3, 116.7, 116.9, 117.0, 117.4, 134.2, 134.5, 134.6, 135.1.

2,3,4,6-Tetra-O-((N-Butoxycarbonylaminooethyl)acetamido)- β -D-galactopyranosyl azide 25

15 Ruthenium trichloride hydrate (20 mg) was added to a vigorously stirred, chilled (0°C) suspension of 2,3,4,6-Tetra-O-allyl- β -D-galactopyranosyl azide 24 (1.18 g, 3.23 mmol) and sodium periodate (11.33 g, 53.0 mmol) in a mixture of tetrachloromethane (20 ml), acetonitrile (20 ml) 20 and water (30 ml). The ice-bath was removed and the mixture was stirred for 3 h, then filtered through a pad of Celite. The filter pad was washed well with methanol, then the combined filtrates were evaporated to give the crude 2,3,4,6-Tetra-O-acetoxy- β -D-galactopyranosyl azide as a 25 yellow foam (1.57 g).

To this crude tetraacid was added N-(butoxycarbonyl)ethylenediamine [Muller, D., Zeltser, I., Bitan, G. and Gilon, C., *J. Org. Chem.*, 1997, **62**, 411-416] (3.10 g, 19.4 mmol), DIEA (4.50 ml, 3.33 g, 25.8 mmol) and 30 N,N-dimethylformamide (40 ml). When all the solids had dissolved, HBTU (4.90 g, 12.9 mmol) was added and the solution was stirred overnight. The solvent was evaporated and the residue was taken up in ethyl acetate (200 ml) and washed with 5% hydrochloric acid (3 x 50 ml), saturated 35 sodium hydrogen carbonate (2 x 50 ml) and brine (50 ml), then dried (MgSO₄) and evaporated to a brown oil. This was dissolved in 10% methanol in ethyl acetate and filtered

- 45 -

through a short silica column. Evaporation of the filtrate gave a brown foam (2.84 g).

MS (electrospray) 1029 ($M+Na$)⁺, 1007 (MH^+), 907 ($M-Boc$)⁺, 806 ($M-2xBoc$)⁺, 706 ($M-3xBoc$)⁺.

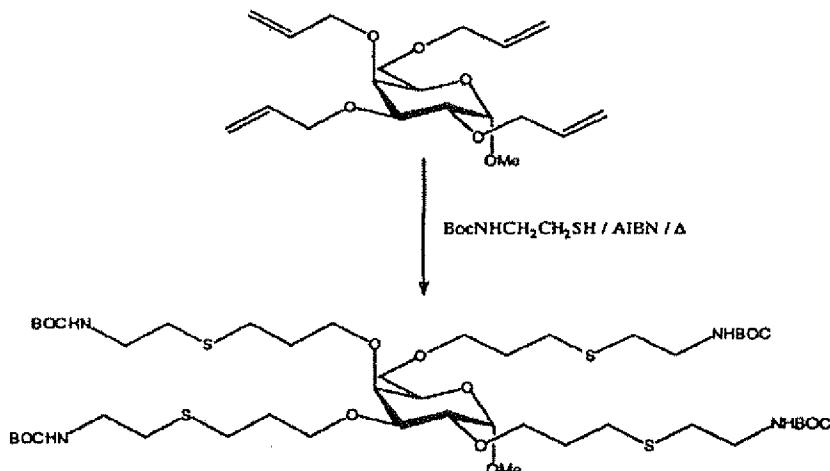
5 R_f 0.53 ethyl acetate-methanol; 9:1

¹H n.m.r. (300 MHz, $CDCl_3$) 1.43, 36H, s, 4 x CH_3 ; 3.00-3.40, 24H, br m, 4 x CH_2O and 8 x CH_2N ; 3.50 - 4.50, 7H, br m, H1, 2, 3, 4, 5, 6_A, 6_B; 5.06, 2H, br s, 2 x NH; 5.40-5.70, 6H, 6 x NH.

10

EXAMPLE 8 PREPARATION OF "SULFIDE" TYPE MATRIX CORE -
LINKER

Scheme 5



15 *Methyl 2,3,4,6-Tetra-O-((N-Butoxycarbonylaminooethylthio)propyl)- α -D-galactopyranoside 26*

A solution of methyl 2,3,4,6-tetra-O-allyl- α -D-glucopyranoside 2 (0.37 g, 1.04 mmol) and N-(tert-butoxycarbonyl)cysteamine (1.48 g, 8.35 mmol) in dioxane (20 ml) was degassed by refluxing for 10 min under an atmosphere of argon. To the cooled solution was added AIBN (10 mg) and the solution was refluxed. After 6 h the solution was diluted with diethyl ether (200 ml) and washed with 10% sodium hydroxide (3 x 100 ml), water (100 ml) and brine (50 ml), then dried ($MgSO_4$) and evaporated to a pale

- 46 -

yellow oil (0.48 g). This was filtered through a short silica column and eluted with 25% diethyl ether in hexane. Evaporation of the filtrate gave a pale yellow oil (0.44 g, 40%).

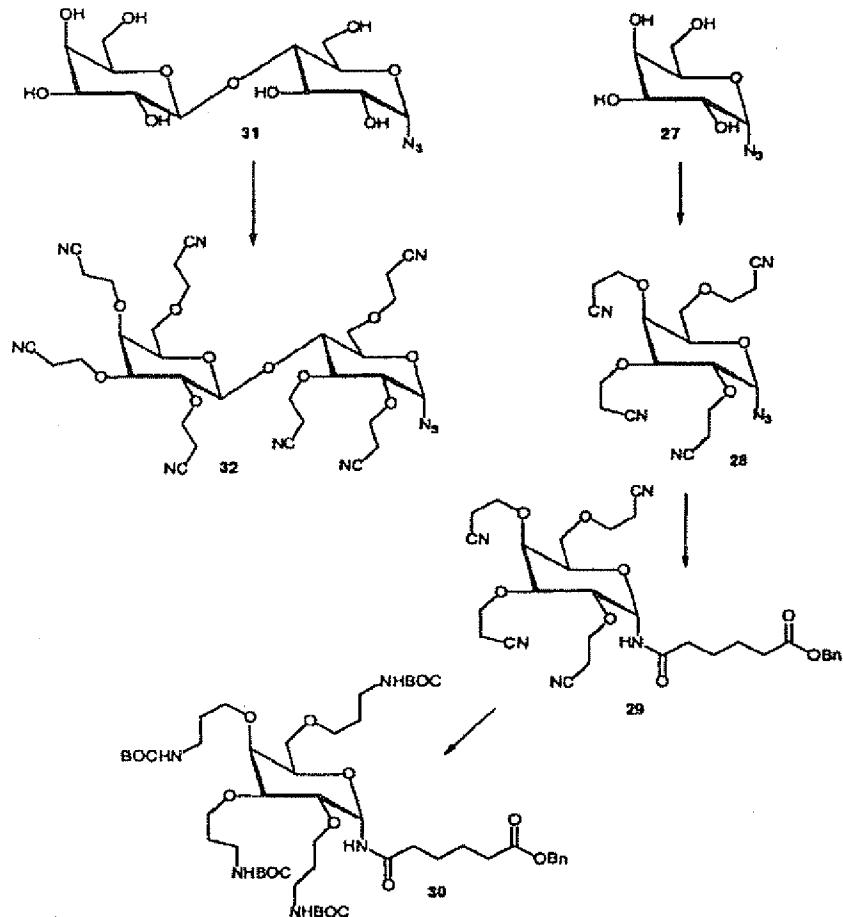
5 MS (electrospray) 1086 (M+Na)⁺, 1064 (MH⁺), 964 (M-Boc)⁺.

R_f 0.40 diethyl ether-hexane; 1:3

¹H n.m.r. (300 MHz, CDCl₃) 0.87, 8H, m, 4 x SCH₂CH₂; 1.40, 36H, 4 x CMe₃; 2.77, 8H, t (J 6.3), 4 x SCH₂;
10 2.90, 8H, t (J 6.5), 4 x SCH₂; 3.28-3.36, 8H, 4 x NCH₂; 3.38-3.45, 11H, OMe and 4 x NCH₂; 4.95, 2H, br s, 2 x NH;
5.17, 2H, br s, 2 x NH.

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EXAMPLE 9 PREPARATION OF AN BOC PROTECTED "ALKYL" TYPE
MATRIX CORE - LINKER AND A DISACCHARIDE
MATRIX CORE.



Scheme 6

Azido 2,3,6,2',3',4',6'-Hepta-O-(2-cyanoethyl)-lactopyranoside 32

10 The lactose azide 31 (2.46 g, 6.3 mmol) was suspended in CH_3CN (50 mL) and acrylonitrile (12.8 mL, 10.4 g, 194 mmol) and DBU (1.2 mL, 1.2 g, 7.8 mmol) were added. The mixture was stirred vigorously. After 5 hours a pale yellow solution was obtained and additional acrylonitrile (12 mL, 0.196 mol) and DBU (1.2 mL, 7.8 mmol) were added. The reaction was monitored by MS at 24 and 48 hours.

- 48 -

Additional acrylonitrile (12.8 mL, 194 mmol) and DBU (1.2 mL, 7.8 mmol) were added after 24 hours. The ES-MS data at 24 and 48 hours were identical.

ES-MS: 757 [M+1]⁺, 703 [(M-CH₂CH₂CN) + 1]⁺

5 The solvent was removed by evaporation and the residue was chromatographed on SiO₂ using hexane-ethyl acetate.

¹H NMR (300 MHz, CDCl₃) 4.54, m; 4.20 - 3.20, overlapping multiplets; 2.60 m.

¹³C NMR (75 MHz, CDCl₃) 118.8, 118.6, 118.4, 10 118.3, 118.0, 117.9, 117.8, 102.0, 89.0, 81.7, 81.6, 78.5, 78.4, 75.1, 74.3, 72.1, 68.1, 68.0, 67.5, 67.4, 66.9, 65.8, 65.7, 66.5, 19.1, 18.9, 18.8, 18.7, 18.6.

In a cognate manner, galactosyl azide 27 was reacted to form the tetra-cyanoethyl derivative 28 in 64% 15 yield.

EXAMPLE 10 PREPARATION OF MATRIX CORE-LINKER-LIPOAMINO ACID CONSTRUCT ON SOLID PHASE

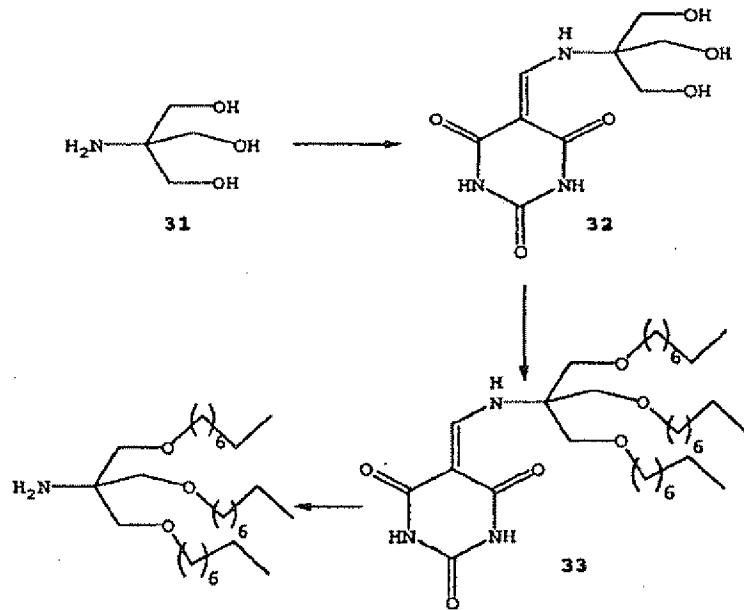
Boc-Gly-MBHA resin is treated with 20 trifluoroacetic acid (neat) for 2 x 1 minute to effect the removal of the Boc group. The resin is washed with DMF and a solution of Boc-lipoaminoacid (4 eq), HOBT (4 eq) and DIPEA (6 eq) is added to the resin. This mixture is shaken for 30 minutes then a sample removed for quantitative ninhydrin. If the coupling has progressed to greater than 25 99%, the resin is drained, washed with DMF then the Boc group is removed by treatment with TFA 2 x 1 minute. A second and third lipoaminoacid is then added and deprotected using the same protocol. If the coupling is 30 less than 99% complete, the resin is drained and resubmitted to the coupling step for a further 30 minutes. If the coupling is still less than 99% after a second coupling, the resin is drained and treated with 10% acetic anhydride in methanol for 5 minutes, drained, washed and 35 the Boc group cleaved. When the construct has the desired number of lipoaminoacids (in this case 3), the terminal Boc is cleaved and a sugar linker conjugate may be added. In

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this example, compound 10 or 18 (4 eq) with HOBT (4 eq) and DIPEA (6 eq) is added and the resin shaken for 30 minutes. Quantitative ninhydrin assay is again used to asses the completion of the reaction and if necessary a second 5 coupling and or capping with acetic anhydride performed.

The phthaloyl groups are then removed from the amino side chains by treatment with hydrazine hydrate as detailed above ad the resin is washed with DMF. A pharmaceutically active agent with a free carboxyl (10 eq), 10 HOBT (10 eq) and DIPEA (16 eq) is then added and the mixture shaken for 2 hours. Quantitative ninhydrin is again used to determine the extent of coupling and if required a second coupling or capping with acetic anhydride is performed. When complete, the resin is washed with DMF, 50% 15 methanol/DCM and finally DCM then air dried before submitting to cleavage using the Hi/Low HF procedure [2].

EXAMPLE 11 PREPARATION OF "TRIS" TYPE LIPIDIC ANCHORS



Scheme 7

- 50 -

2-(1,3-dimethyl-2,4,6(1H,3H,5H)-trioxopyrimidine-5-ylidene)methylamino-2-hydroxymethyl-1,3-propanediol 2 (32)

Sodium (729 mg, 31.725 mmol) in anhydrous methanol (30 mL) was stirred with 2-amino-2-hydroxymethyl-
5 1,3-propanediol hydrochloride 31 (5.0 g, 31.725 mmol) for 10 minutes at 30 °C. 1,3-Dimethyl-5-[(dimethylamino)methylene] 2,4,6(1H,3H,5H)-trioxopyrimidine (7.5 g, 33.31 mmol) was dissolved in methanol (20 mL) and added in one portion. The product 10 precipitated immediately. It was filtered and washed on the funnel with cold methanol (50 mL) and cold ether (2 x 50 mL). The product was dried *in vacuo* over P₂O₅ and not further purified to yield 2-(1,3-dimethyl-2,4,6(1H,3H,5H)-trioxopyrimidine-5-ylidene)methylamino-2-hydroxymethyl-1,3-propanediol 32 (8.84 g 97 %).

R_f 0.5, 10-1 Acetonitrile-H₂O (Vanillin stain)

MS C₁₁H₁₇N₃O₆ (288.1) m/z [M+H]⁺

2-(1,3-dimethyl-2,4,6(1H,3H,5H)-trioxopyrimidine-5-ylidene)methylamino-2-octyloxymethyl-1,3-octyloxypropane 33

2-(1,3-Dimethyl-2,4,6(1H,3H,5H)-trioxopyrimidine-5-ylidene)methylamino-2-hydroxymethyl-1,3-propanediol 32 (250 mg, 0.87 mmol) was dissolved in anhydrous DMF (10 mL) and stirred with NaH (140 mg (60 % oil dispersion), 3.5 mmol) for one hour. 1-Bromooctane (0.6 mL, 3.5 mmol) was added and after 4 hours additional NaH (140 mg (60% oil dispersion, 3.5 mmol) and 1-bromooctane (0.6 mL, 3.5 mmol) were added. The resulting mixture was stirred overnight at room temperature. Methanol (5 mL) was added dropwise and 30 the solvent azeotropically removed with toluene (2 x 20 mL). The crude residue was dissolved in CHCl₃ (20 mL), washed with water (3 x 20 mL) and saturated brine solution (20 mL), dried over MgSO₄, filtered and evaporated to dryness under reduced pressure. Purification by silica gel chromatography (Hexane-EtOAc (4-1)) gave 2-(1,3-dimethyl-2,4,6(1H,3H,5H)-trioxopyrimidine-5-ylidene)methylamino-2-

- 51 -

octyloxymethyl-1,3-octyloxypropane **33** as a pale yellow oil (270 mg, 49.7 %).

R_f 0.8 10-1 DCM-MeOH, (Vanillin stain)

MS $C_{35}H_{65}N_3O_6$ (624.57) m/z $[M+H]^+$

5

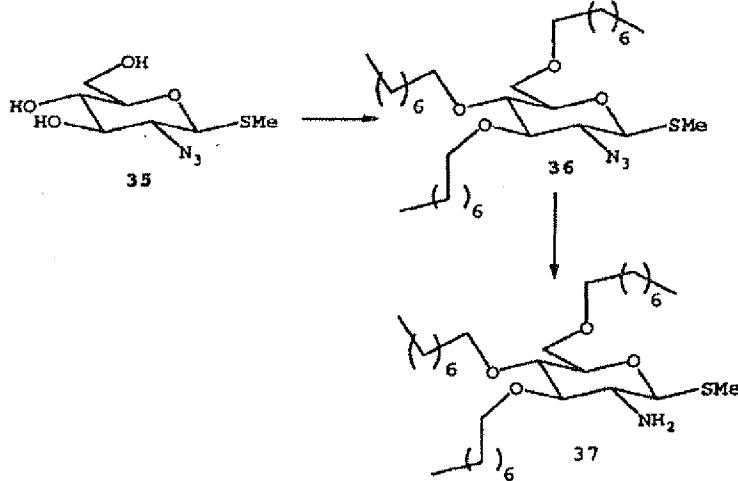
2-amino-2-octyloxymethyl-1,3-octyloxypropane 34

To a solution of 2-(1,3-dimethyl-2,4,6(1H,3H,5H)-trioxopyrimidine-5-ylidene)methylamino-2-octyloxymethyl-1,3-octyloxypropane **33** (210 mg, 0.337 mmol) in ethanol (12 mL), was added Hydrazine hydrate (0.5 mL). The reaction mixture was stirred at room temperature for 15 minutes. After which time a precipitate had formed. The reaction mixture was filtered through celite. The filter was washed with cold methanol (10 mL) and the filtrate evaporated to dryness under reduced pressure. Purification by silica gel chromatography (dichloromethane then dichloromethane-MeOH (10/1)) gave 2-methylamino-2-octyloxymethyl-1,3-octyloxypropane **34** (139 mg, 90%).

R_f 0.7 10-1 DCM-MeOH, (Vanillin stain)

20 MS $C_{28}H_{59}NO_3$ (458.55) m/z $[M+H]^+$

EXAMPLE 12 PREPARATION OF A "CARBOHYDRATE" TYPE LIPIDIC ANCHOR



25

Scheme 7

- 52 -

Methyl 2-azido-2-deoxy-3,4,6-tri-O-octyl-1-thio- β -D-glucopyranoside 36

Methyl 2-azido-2-deoxy-1-thio- β -D-glucopyranoside 35 (1.0 g, 4.255 mmol) was dissolved in anhydrous DMF (50 mL). NaH (0.68 g (60 % oil dispersion), 17.02 mmol) was added to the mixture and stirred for 10 minutes, followed by 1-bromooctane (5.15 mL, 29.78 mmol). Additional NaH (0.3 g (60% oil dispersion), 7.5 mmol) and 1-bromooctane (2.5 mL, 14.47 mmol) were added after three hours and the reaction left overnight. Methanol was added dropwise (5 mL) and then the solvent azeotropically removed with toluene (2 x 30 mL). The crude product was dissolved in ethyl acetate (100 mL), washed with water (2 x 100 mL) and saturated brine solution (2 x 100 mL), dried over MgSO₄, filtered and evaporated to dryness. Silica gel chromatography (petroleum ether-ethyl acetate (20-1.5)) yielded methyl 2-azido-2-deoxy-3,4,6-tri-O-octyl-1-thio- β -D-glucopyranoside 36 as yellow oil (550 mg, 23%).

R_f 0.45 20-1.5 PE-EtOAc

20 MS C₃₁H₆₁N₃O₄S (570.6) m/z [M-H]⁺

Methyl 2-amino-2-deoxy-3,4,6-tri-O-octyl-1-thio- β -D-glucopyranoside 37

Methyl 2-azido-2-deoxy-3,4,6-tri-O-octyl-1-thio- β -D-glucopyranoside 36 (200 mg, 0.349 mmol), DTT (110 mg, 0.699 mmol) and dry triethylamine (97 μ L, 0.699 mmol) were dissolved in dry DMF (2 mL) under nitrogen. Nitrogen was bubbled in to the reaction mixture for 30 minutes, followed by overnight stirring at room temperature. Additional DTT (110 mg, 0.699 mmol) and dry triethylamine (97 μ L, 0.699 mmol) were added and stirring continued for 24 hours. The solvent was azeotropically removed with acetonitrile (3 x 10 mL) and the residue dissolved in CHCl₃ (20 mL). The organic phase was washed with water (6 x 20 mL), dried over MgSO₄, filtered and evaporated to dryness, quantitatively yielding methyl 2-amino-2-deoxy-3,4,6-tri-O-octyl-1-thio- β -

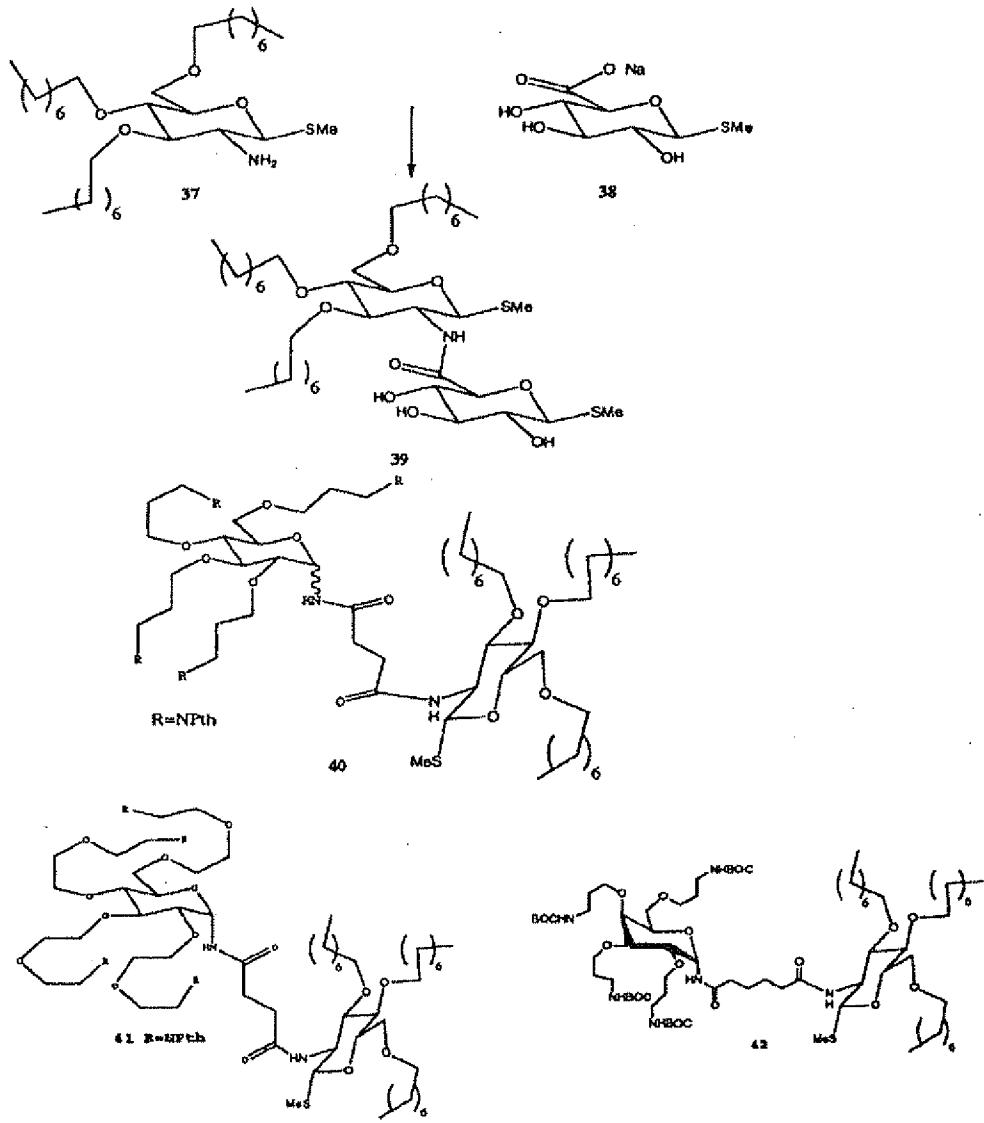
- 53 -

D-glucopyranoside **37** as an oily solid that was not further purified.

R_f 0.15 6:1 hexane-ethyl acetate

5 EXAMPLE 13

PREPARATION OF ALTERNATIVE MATRIX CORE -
LINKER - LIPIDIC COMPONENTS



Scheme 8

- 54 -

Methyl 2-(methyl 1-thio- β -D-glucuron)amido-2-deoxy-3,4,6-tri-O-octyl-1-thio- β -D-glucopyranoside 39

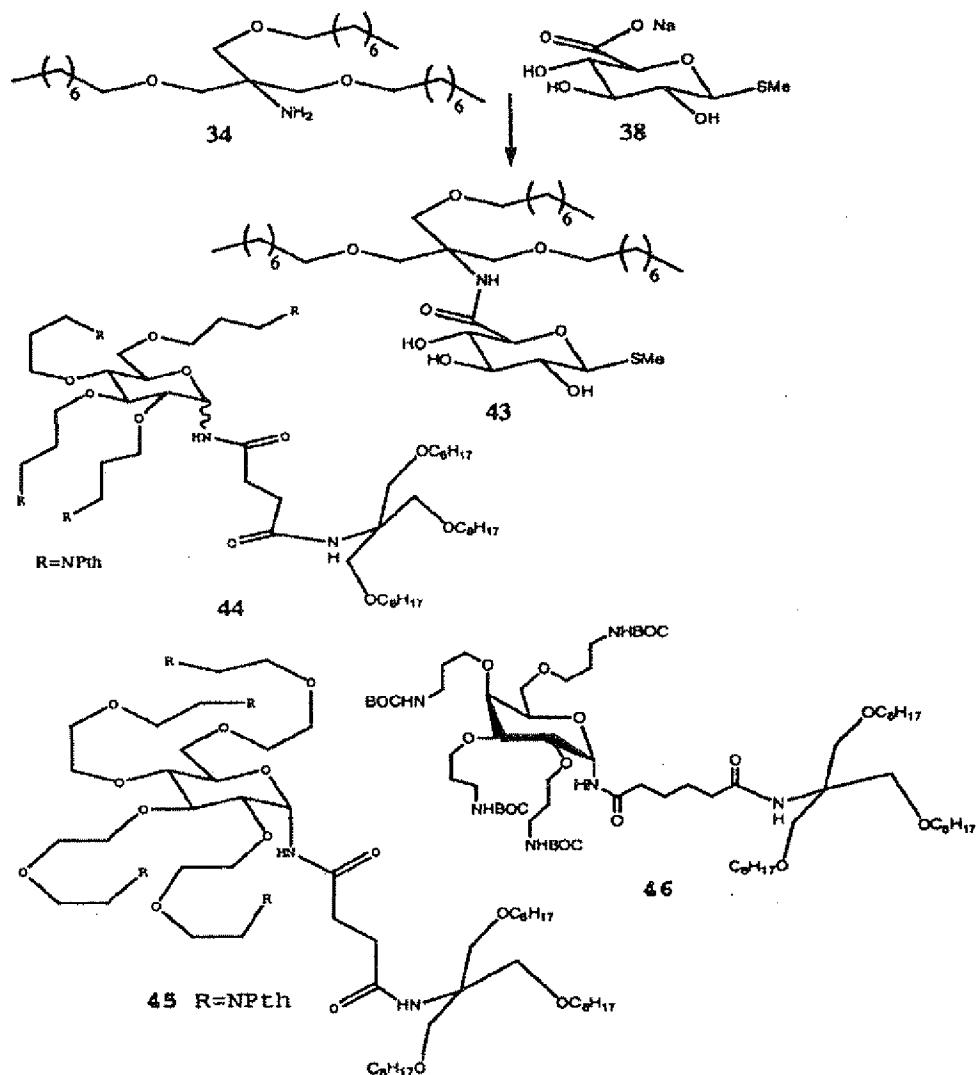
Sodium [methyl (1-thio- β -D-glucopyranosid)uronate 38 (10 mg, 0.0408 mmol), methyl 2-amino-2-deoxy-3,4,6-tri-O-octyl-1-thio- β -D-glucopyranoside 37 (23 mg, 0.0421 mmol) and HBTU (86 μ L, 0.5 M in DMF) were dissolved in anhydrous DMF (0.2 mL). DIPEA (20 μ L, 0.105 mmol) was added and the mixture left at room temperature overnight. The solution was diluted with CHCl₃ (7 mL) and washed with 10 % citric acid solution (2 x 20 mL), saturated NaHCO₃ solution (2 x 20 mL) and saturated brine solution (2 x 20 mL). The aqueous layers were back-extracted with CHCl₃ (20 mL) and the organic layers combined, dried over MgSO₄, filtered and evaporated to dryness. The crude residue was purified by silica gel chromatography, using CHCl₃ to CHCl₃-MeOH (10-1) as the mobile phase. Methyl 2-(methyl 1-thio- β -D-glucuron)amido-2-deoxy-3,4,6-tri-O-octyl-1-thio- β -D-glucopyranoside 39 was isolated a white film (11 mg, 36%).

20 R_f 0.45 10-1 CHCl₃-MeOH

MS C₃₈H₇₃NO₉S₂ (752.68) m/z [M+H]⁺

In a cognate manner, compounds 40, 41 and 42 can be prepared by reaction of the lipidic anchor 37 with matrix-core - linker components 10, 18 and 30 respectively 25 under the same conditions.

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Scheme 9

Methyl [6-(2-methylamino-2-octyloxymethyl-1,3-octyloxyp propane)-1-thio- β -D-glucopyranosid]uronate 43

2-methylamino-2-octyloxymethyl-1,3-octyloxyp propane 34 (25 mg, 0.055 mmol), sodium [methyl (1-thio- β -D-glucopyranosid)uronate 38 (12.7 mg, 0.052 mmol) and DIPEA (18 mg, 0.14 mmol) were dissolved in anhydrous DMF (0.2 mL) and anhydrous toluene (25 μ L). HBTU (100 μ L, 0.5 M in DMF) was added and the mixture left at room temperature for 72 hours. The solution was diluted with DCM

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(5 mL) and washed with water (5 mL), 5% citric acid solution (5 mL), saturated NaHCO₃ solution (5 mL) and saturated brine solution (5 mL). The aqueous layers were back-extracted with DCM (5 mL) and the organic layers 5 combined, dried over MgSO₄, filtered and evaporated to dryness. The crude residue was purified by silica gel chromatography, using CHCl₃ to CHCl₃-MeOH (10-1) as the mobile phase. The product was isolated a white film (8 mg, 23%).

10 R_f 0.4 10-1 CHCl₃-MeOH

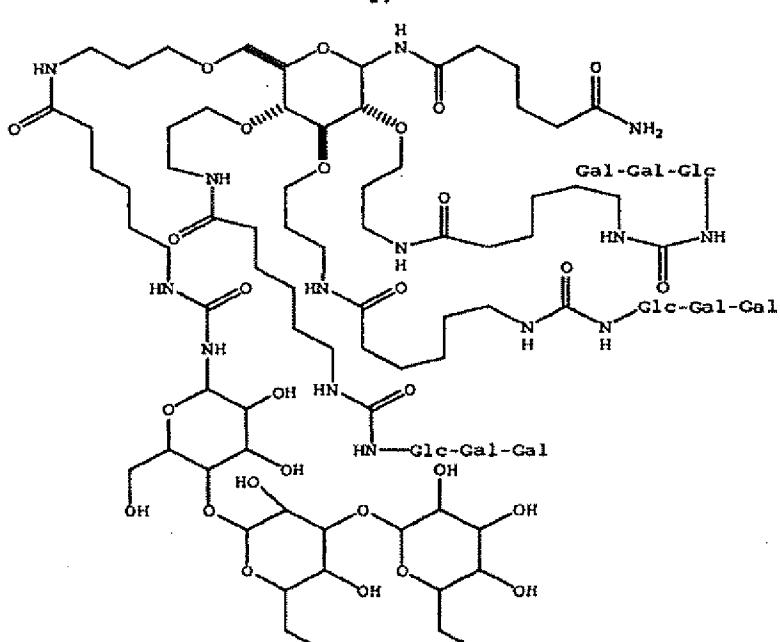
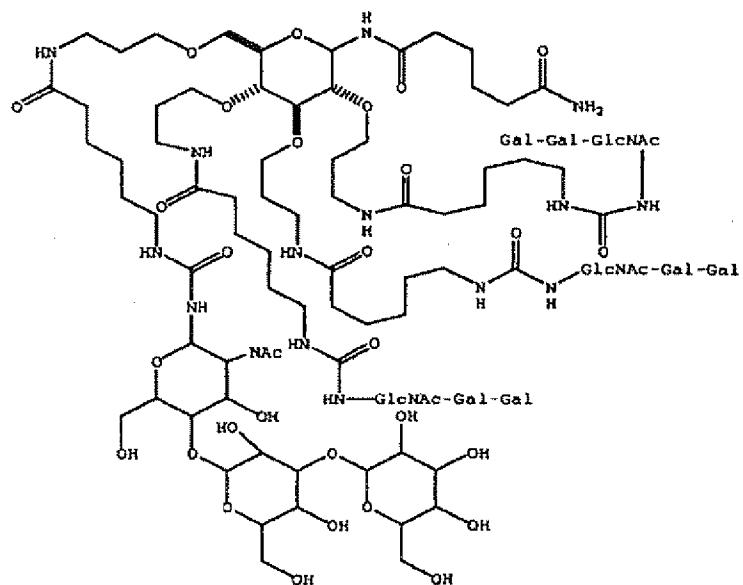
MS C₃₅H₆₉NO₈S (664.6) m/z [M+H]⁺

15 In a cognate manner, compounds 44, 45 and 46 can be prepared by reaction of the lipidic anchor 34 with matrix-core - linker components 10, 18 and 30 respectively under the same conditions.

EXAMPLE 14 PREPARATION OF LIGAND - LINKER - MATRIX
CORE CONSTRUCTS

20 Gal- α -1,3-Gal- β -1,4-Glc and Gal- α -1,3-Gal- β -1,4-GlcNAc are carbohydrate epitopes present on the surface of non-human cells and are responsible for the recognition of xenobiotic invasion. Constructs containing these epitopes may be pharmacologically useful in for example sequestering 25 antibodies, eliciting an antibody response or binding other agents which are reactive with these epitopes, for example *clostridium difficile* derived toxin a and toxin b.

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Preparation of compound 47 and 48

5 *N,N-Diisopropylethylamine (48 µl, 35 mg, 0.27 mmol) was added to a stirred suspension of the Glucosyl-linker-carboxylic acid 30 (128 mg, 0.136 mmol), HBTU (57*

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mg, 0.15 mmol) and ammonium chloride (15 mg, 0.27 mmol) in tetrahydrofuran (2.0 ml). After stirring overnight, the mixture was diluted with ethyl acetate (100 ml) and washed with 5% hydrochloric acid (2 x 10 ml), saturated NaHCO₃ (2 x 10 ml), water (10 ml) and brine (10 ml), then dried (MgSO₄) and evaporated to give the amide as a colorless gum (121 mg, 94%).

ES-MS: 936 (MH)⁺, 958 (M+Na)⁺, 974 (M+K)⁺

¹H NMR (300 MHz, CDCl₃) δ 1.40, br s, 36H, 4xCMe₃;
10 1.50-1.80, m, 12H, 6xCH₂; 2.20-2.35, m, 4H, 2xCH₂CO; 3.10-
3.80, m, 22H, 4xCH₂N, 5xCH₂O and 4xCHO; 4.90-5.20, m, 7H,
7xNH.

¹³C NMR (75 MHz, CDCl₃) δ 24.5, 24.6, 24.8, 25.0,
28.4, 29.6, 30.4, 30.6, 35.2, 35.3, 35.8, 37.9, 38.2, 38.4,
15 65.1, 67.9, 69.3, 69.8, 70.0, 71.2, 75.6, 76.1, 78.9, 79.1,
79.4, 79.5, 156.0, 156.1, 156.6, 174.0, 176.0.

The protected glucosyl-linker-amide above (6.9 mg, 7.4 μmol) was dissolved in CH₂Cl₂ (1.0 ml). Trifluoroacetic acid (1.0 ml) was added, and the solution 20 was allowed to stand for 5 min before being evaporated, then coevaporated with several portions of CH₂Cl₂. The residue was then lyophilised three times from 1:1 acetonitrile water. The trisaccharide (Galα(1→3)Galβ(1→4)GlcNAc-NHCONH(CH₂)₅CO₂H) (28 mg, 40 25 μmol) was added, together with HBTU (23 mg, 61 μmol) and DMF (2.0 ml). Diisopropylethylamine (71 μl, 53 mg, 400 μmol) was added, and the solution was stirred over the weekend, then evaporated. The product was purified by HPLC (acetonitrile/water containing 0.1% formic acid) and 30 lyophilised to give the product 47 as a colorless powder.

ES-MS: 1635 (MH₂)²⁺

¹H NMR (300 MHz, d₆-DMSO) (inter alia) 1.50-1.80, m, 28H, 14xCH₂; 2.20-2.40, m, 12H, 6xCH₂CO; 2.90, br s, 12H, 4x AcNH; 3.05-3.90, m, 137H, 8xCH₂N, 17xCH₂O, 51xCHO and 35 36xOH; 4.80-5.40, m, 19H, 19xNH.

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In a cognate manner compound **48** was prepared by substituting (Gal α (1 \rightarrow 3)Gal β (1 \rightarrow 4)Glc-NHCONH(CH₂)₅CO₂H for (Gal α (1 \rightarrow 3)Gal β (1 \rightarrow 4)GlcNAc-NHCONH(CH₂)₅CO₂H).

Characterisation data for **48** are as follows:

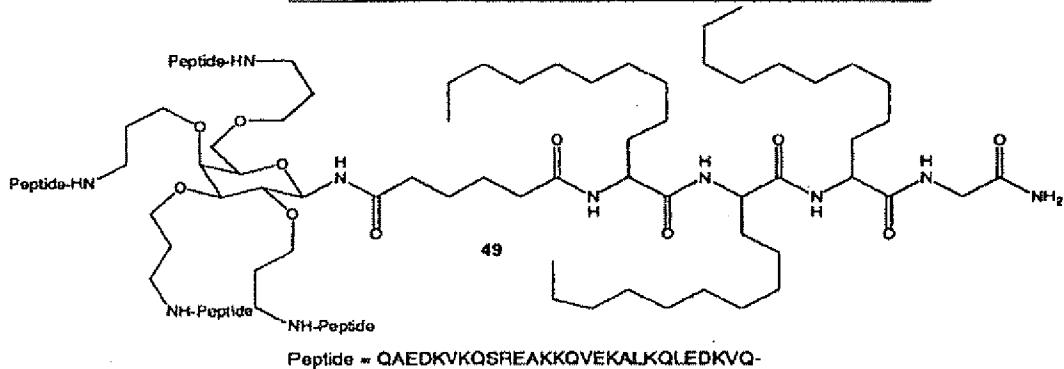
5 ES-MS: 1553 (MH₂)²⁺

10 ¹H NMR (300 MHz, d₆-DMSO) (inter alia) 1.50-1.80, m, 28H, 14xCH₂; 2.20-2.40, m, 12H, 6xCH₂CO; 3.05-3.90, m, 141H, 8xCH₂N, 17xCH₂O, 51xCHO and 40xOH; 4.80-5.40, m, 15H, 15xNH.

10

EXAMPLE 15

PREPARATION OF LIGAND - LINKER - MATRIX CORE -LINKER -LIPIDIC ANCHOR CONSTRUCTS.



*Preparation of compound **49***

15

Boc - Glycine was coupled to MBHA resin using standard peptide coupling procedures outlined above. Deprotection of the BOC group and subsequent coupling of three units of 2-aminododecanoic acid through standard BOC/HBTU chemistry outlined above, yielded the lipidic anchor on resin. Compound **30** was then coupled after hydrogenolysis of the benzyl ester, to yield the linker -core matrix - linker -lipidic anchor construct. The Boc groups were removed with TFA and the peptide sequence synthesized on this construct by sequential HBTU couplings and BOC deprotections. Finally the construct was cleaved from the resin by HF-cleavage as outlined above, and after workup compound **49** was isolated. The construct was characterized by mass spectroscopy m/z 14300 M+H reconstruct.

25

- 60 -

Serum IgG antibody responses to this construct were measured in groups of mice. Antibody titres were compared with those observed for the free peptide and the LCP system (lipoaminoacid - polylysine system). In all 5 cases, the antigen was injected with complete freunds adjuvant. Mice were dosed at day 1 with subsequent boosts at days 7 and 14. Antibody titres were determined after 21 days.

Raw data indicates that the free peptide/complete 10 freunds adjuvant system elicited a titre of approximately 10^4 antibodies, the peptide-LCP/complete freunds adjuvant system elicited a titre of approximately 10^6 antibodies, and compound 49/complete freunds adjuvant elicited a titre of 3×10^6 antibodies. Although preliminary, these 15 experiments indicate that compound 49 elicits an increased immunological response over the free peptide. Further immunological work is ongoing with this and other constructs.

It will be apparent to the person skilled in the 20 art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this 25 specification.

References cited herein are listed on the following page, and are incorporated herein by this reference.

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CLAIMS:

1. A compound of general formula I

5

$(A-L)_n-S-R'$

I

in which S is a carbohydrate core matrix;
10 A is a pharmaceutically active moiety;
L is a linker or covalent bond;
n is an integer of 2 to 19; and
R' is hydrogen, thioalkyl or -X-Z in which Z is a
lipophilic anchor and X is a linker or covalent bond.

15

2. A compound according to claim 1 in which the carbohydrate core matrix S is a natural or synthetic monosaccharide or an oligosaccharide.

20

3. A compound according to claim 2, wherein the carbohydrate core matrix S an oligosaccharide with 1 to 6 monosaccharide moieties.

25

4. A compound according to claim 2, wherein the carbohydrate core matrix S is selected from the group consisting of glucose, glucosamine, galactose, mannose, glucuronic acid, iduronic acid, idose, fucose, galactosamine, sucrose, fructose, maltose, lactose, lactosamine, globotriose, globotetraose, sialyl lewis X, lewis X, lewis Y, lewis b tetrasaccharide, lewis a, sialyl lewis a, chitobiose, chitotriose, chitotetraose, chitopentaose, chitohexaose, blood group A trisaccharide, blood group b trisaccharide, blood group H disaccharide, blood group H II trisaccharide, galabiose, T antigen, $\alpha 1,3$ galactobiose, Gal $\alpha 1,3$ Gal $\beta 1,4$ GlcNAc, Galili, pentasaccharide, mannobiose, 3'-sialyllactose, 6'-sialyllactose, sialyl lacto-N-tetraose, lacto-N-tetraose,

lacto-N-neotetraose, lacto-N-hexaose, lacto-N-neohexaose, cellobiose, cellopentaose, cellotetraose, cellotriose, α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin, ribose, arabinose, trigalacturonic acid and maltotriose.

5

5. A compound according to claim 1 in which the pharmaceutically-active moiety A is selected from synthetic or natural peptides, proteins, mono- or oligosaccharides, sugar-amino acid conjugates, sugar-peptide conjugates, 10 drugs, pro-drugs or drug like molecules.

6. A compound according to any one of claims 1 to 5 in which the pharmaceutically-active moiety A serves as an antigen or antigenic determinant, toxin, ligand, drug or 15 pro-drug and is either antigenic or non-antigenic when taken alone.

7. A compound according to any one of the preceding claims in which the linker L is alkyl, alkenyl, alkynyl, 20 heteroalkyl, arylalkyl or heteroarylalkyl of 3 to 14 atoms in length which is optionally substituted, branched and/or linear.

8. A compound according to any one of the preceding 25 claims in which the linker X is:

- (a) a monosaccharide or an oligosaccharide having 1 to 4 monosaccharide moieties;
- (b) a spacer of up to 14; or
- (c) a peptide or amino acid.

30

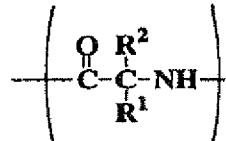
9. A compound according to claim 8, in which the spacer (b) is an alkyl, alkenyl, alkynyl, heteroalkyl, arylalkyl or heteroarylalkyl, each of which is optionally substituted.

35

10. A compound according to claim 8 or claim 9, in which the spacer is polyethyleneglycol or polyglycine.

11. A compound according to any one of the preceding claims in which the lipophilic anchor Z is:

(a) 1 to 6 lipoamino acids of general formula II

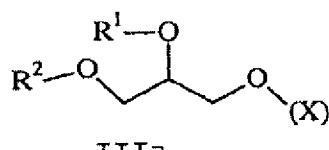


5

II

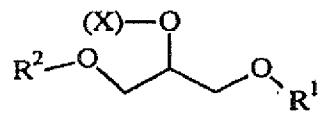
in which each of R^1 and R^2 are the same or different and selected from hydrogen or an alkyl or alkenyl having 4 to 24 carbon atoms, each of which are optionally substituted with substituents that do not significantly adversely affect the lipophilic nature of the anchor, with the proviso that both R^1 and R^2 are not hydrogen;

15 (b) a glycerol based lipid of the general formula IIIa or IIIb



20

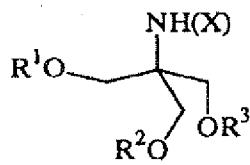
IIIa



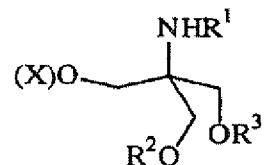
IIIb

in which R^1 and R^2 are as defined in formula II above and X is the linker defined in claim 1;

25 (c) a trishydroxymethylmethylamine-based lipid of general formula IVa or IVb



IVa

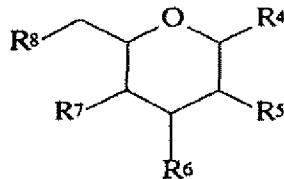


IVb

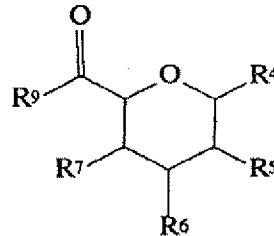
5 in which R^1 , R^2 and R^3 are the same or different and selected from hydrogen or a group having 4 to 24 carbon atoms which is optionally substituted, with substituents do not significantly adversely affect the lipophilic nature of the anchor; and

10 X is the linker as defined in claim 1, with the proviso that only one of R^1 , R^2 and R^3 can be hydrogen; or

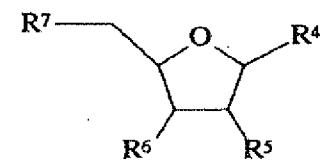
• (d) a compound of general formula Va, Vb or Vc



Va



Vb



Vc

15

in which the groups R^4 , R^5 , R^6 , R^7 and R^8 are the same or different and selected from $O(X)$, $NH(X)$, OH , OW , $N-(C=O)-W$ and NH_2 ,

20 W is a alkyl or alkenyl group having 4 to 24 carbon atoms which may be optionally substituted with substituents do not significantly adversely affect the lipophilic nature of the anchor, (X) is the linking group to the sugar core,

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with the provisos that at least two of the groups R⁴ to R⁸ must be OW and only one of the groups may be O(X) or NH(X).

in which R⁹ is selected from OW, (X), O(X), NH-W,
5 NH-(X), OH or NH₂ where W is as defined above, (X) is the linking group to the sugar core, with the proviso that R⁹ may only be (X), O(X) or NH-(X) if none of R⁴, R⁵, R⁶, R⁷ or R⁸ are O(X) or NH(X).

additionally, R⁴ may be thioalkyl.

10

12. A compound according to any one of the preceding claims which is of the formula Ia

(A-L)_n-S-X-Z

15

Ia

in which S, A, L, n, X and Z are as defined in any one of the preceding claims.

20

13. A compound according to any one of claims 1 to 11, which is of the formula Ib

(A-L)_n-S

25

Ib

in which A, L and S are as defined in any one of claims 1 to 11; and

30

n is an integer of 2 to 11.

14. A method for the preparation of a compound of formula I as defined in any one of the preceding claims, comprising the steps of

35

(a) attaching the linker L to the core matrix S;
(b) attaching a pharmaceutically active moiety A to the linker L; and

when R' is -X-Z

(c) linking the lipophilic anchor Z and the core matrix S either by (i) the reaction of a glycosylamine with the carboxylate terminus or amine of the lipophilic anchor,
5 (ii) the reaction of a glycosylamine with the carboxylate terminus of the lipophilic acid and linker X to form a peptide bond, (iii) glycosylating the core matrix S via the lipophilic anchor Z or the linker X to form an N, O, or S glycosidic bond and/or (iv) reacting a glycosyl carboxylate
10 either directly or via a linker X with an amine of the lipophilic anchor Z to form a peptide bond.

15. A compound of the general formula Va, Vb or Vc as defined in claim 11.

15

16. A compound according to any one of claims 1 to 15, together with a pharmaceutically-acceptable carrier.

20

17. A method of treating or preventing a disease comprising the step of administering to a subject in need thereof a therapeutically-effective amount of a compound according to any one of claims 1 to 16.

25

18. A method of immunisation for the treatment or prophylaxis of a disease, comprising the step of administering to a subject in need thereof a immunologically-effective amount of a compound according to any one of claims 1 to 16.

30

19. A method according to claim 17 or claim 18, wherein the subject is selected from the group consisting of dogs, cats, horses, cattle and sheep or other mammals.

35

20. A method according to claim 17 or claim 18, wherein, the subject is a human.

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21. A method according to any one of claims 17 to 20, wherein the compound is administered prior to, together with, or subsequent to the administration of a pharmaceutically-active agent.

5

22. A method according to any one of claims 17 to 21, wherein the compound is administered together with a pharmaceutically acceptable carrier, solvent, suspending agent or vehicle.

10

23. A method according to claim 22, wherein the carrier is a liquid carrier.

15

24. A method according to claim 22, wherein the carrier is a solid carrier.

25. A method according to any one of claims 17 to 24, wherein the compound is administered orally, topically, or parenterally.

20

26. A method according to claim 25, wherein the parenteral administration is via subcutaneous injections, aerosol, intravenous, intramuscular, intrathecal, intracranial, injection or infusion.

25

27. A method according to claim 22, wherein the solvent is selected from the group consisting of propylene glycol, polyethylene glycol, vegetable oils and injectable organic esters.

30

28. A method according to claim 22, wherein the carrier is water, alcoholic/aqueous solutions, emulsion or suspension.

- 70 -

29. A method according to claim 22, wherein the vehicle is a parenteral vehicle selected from the group consisting of sodium chloride solution, Ringer's dextrose, dextrose, lactated Ringer's intravenous vehicles and 5 electrolyte replenisher.

30. A method according to any one of claims 17 to 29, wherein the disease is either a neoplasm, a cancer, fibrotic disorder, Alzheimer's disease, dementia, motor 10 neurone disease, cardiovascular disease, congestive heart failure, hypertension, hormonal imbalance, atherosclerosis, disorder of development and growth or disorder of glucose and fat metabolism.

15 31. A method according to any one of claims 17 to 30, wherein the compound is administered in the form of liposomes.

20 32. A method according to claim 31, wherein the liposomes are either small unilamellar vesicles, large unilamellar vesicles, or multilamellar vesicles.

AMENDED CLAIMS

[received by the International Bureau on the 2nd april 2002 (02.04.02)]

claims 1-32, replaced by new claims 1-31; claim 15 deleted.

when R' is -X-Z

(c) linking the lipophilic anchor Z and the core matrix S either by (i) the reaction of a glycosylamine with the carboxylate terminus or amine of the lipophilic anchor,
5 (ii) the reaction of a glycosylamine with the carboxylate terminus of the lipophilic acid and linker X to form a peptide bond, (iii) glycosylating the core matrix S via the lipophilic anchor Z or the linker X to form an N, O, or S glycosidic bond and/or (iv) reacting a glycosyl carboxylate either directly or via a linker X with an amine of the
10 lipophilic anchor Z to form a peptide bond.

15. A compound according to any one of claims 1 to 14, together with a pharmaceutically-acceptable carrier.

15 16. A method of treating or preventing a disease comprising the step of administering to a subject in need thereof a therapeutically-effective amount of a compound according to any one of claims 1 to 15.

20 17. A method of immunisation for the treatment or prophylaxis of a disease, comprising the step of administering to a subject in need thereof a immunologically-effective amount of a compound according to
25 any one of claims 1 to 15.

18. A method according to claim 16 or claim 17, wherein the subject is selected from the group consisting of dogs, cats, horses, cattle and sheep or other mammals.

30 19. A method according to claim 16 or claim 17, wherein, the subject is a human.

20. A method according to any one of claims 16 to 19,
35 wherein the compound is administered prior to, together with, or subsequent to the administration of a pharmaceutically-active agent.

21. A method according to any one of claims 16 to 20, wherein the compound is administered together with a pharmaceutically acceptable carrier, solvent, suspending agent or vehicle.

5

22. A method according to claim 21, wherein the carrier is a liquid carrier.

10 23. A method according to claim 21, wherein the carrier is a solid carrier.

15 24. A method according to any one of claims 16 to 23, wherein the compound is administered orally, topically, or parenterally.

20 25. A method according to claim 24, wherein the parenteral administration is via subcutaneous injections, aerosol, intravenous, intramuscular, intrathecal, intracranial, injection or infusion.

25 26. A method according to claim 21, wherein the solvent is selected from the group consisting of propylene glycol, polyethylene glycol, vegetable oils and injectable organic esters.

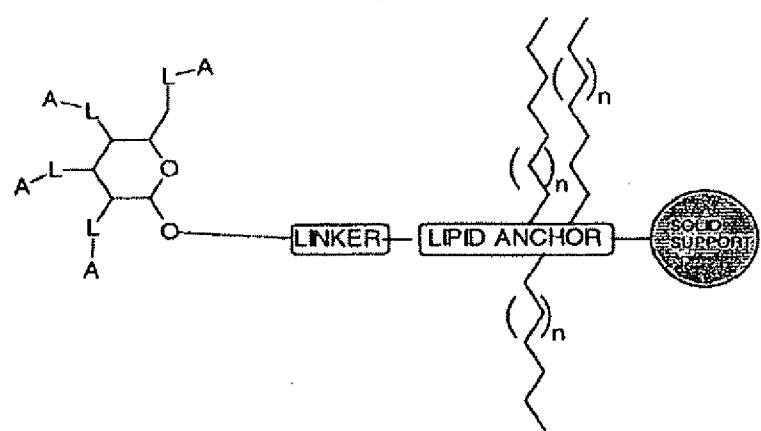
30 27. A method according to claim 21, wherein the carrier is water, alcoholic/aqueous solutions, emulsion or suspension.

35 28. A method according to claim 21, wherein the vehicle is a parenteral vehicle selected from the group consisting of sodium chloride solution, Ringer's dextrose, dextrose, lactated Ringer's intravenous vehicles and electrolyte replenisher.

29. A method according to any one of claims 16 to 28,
wherein the disease is either a neoplasm, a cancer,
fibrotic disorder, Alzheimer's disease, dementia, motor
neurone disease, cardiovascular disease, congestive heart
failure, hypertension, hormonal imbalance, atherosclerosis,
5 disorder of development and growth or disorder of glucose
and fat metabolism.

30. A method according to any one of claims 16 to 29,
10 wherein the compound is administered in the form of
liposomes.

31. A method according to claim 30, wherein the
liposomes are either small unilamellar vesicles, large
15 unilamellar vesicles, or multilamellar vesicles.

I/I*Figure 1*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01313

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.?: C07K 17/00, 17/02, 17/06; C07H 15/04, 17/04; A61K 47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: FILE CA Substructure Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Polysaccharides in Medicinal Applications. Edited by Severian Dumitriu. Published by Marcel Dekker, Inc. New York. 1996. ISBN 0-8247-9540-7 Pages 717-730. See especially page 725	1-14, 16-32
X	ALBERTS, Bruce et al. Molecular Biology of the Cell. Third Edition. Garland Publishing, Inc. New York 1994. ISBN 0-8153-1619-4. Pages 166-169 See pages 167-168	1-14, 16-32
X	ROITT, Ivan et al. Immunology. Fourth Edition. Published by Mosby, an imprint of Times Mirror International Publishers Limited London 1996. ISBN 0-7234-2178-1 pages 28.7-28.8	1-14, 16-32

 Further documents are listed in the continuation of Box C See patent family annex

• Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 2 November 2001	Date of mailing of the international search report - 2 FEB 2002
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer CHRISTINE BREMERS Telephone No : (02) 6283 2313

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01313

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01313

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos :
because they relate to subject matter not required to be searched by this Authority, namely:
2. Claims Nos :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The claims are broadly drafted such that a complete search was not economically feasible.
The search was limited as follows: Claim 1 was limited to the examples.
Claim 15 was limited to where R6 and R7 on Va and Vb and R6 on Vc are O-alkyl.
3. Claims Nos :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. Claims 1-14 and 16-32 (in part) are directed to pharmaceutical compounds of general formula I and to methods of treating or preventing disease using them.
2. Claims 15 and 16-32 (in part) are directed to lipophilic anchor compounds of formulas Va, Vb and Vc as defined in claim 11(d) and methods of treating or preventing disease using Va, Vb or Vc.
1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/01313

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member		
WO 9201702	US 5206396	US 5236909	
END OF ANNEX			